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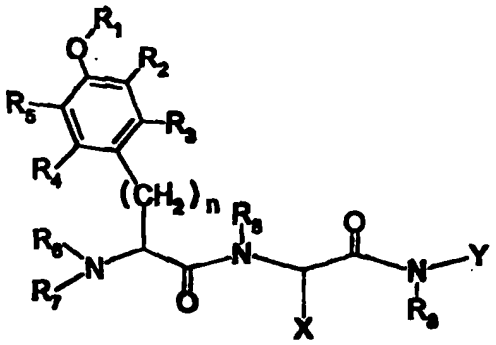
(54) Title: ANALGESIC PEPTIDOMIMETIC COMPOUNDS

(57) Abstract

The present invention is directed to compounds which exhibit analgesic activity. More specifically, the analgesic compounds of the invention are peptidomimetic compounds which can bind to opioid receptors having formula (I) wherein $R_1$ to $R_8$, X and Y are as defined herein.



(I)

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Previously, opiates, opioid peptides, and analogs thereof, have either failed to demonstrate, or have demonstrated only a limited degree of specificity and selectivity for the type of receptor, or receptors, to which they bind.

The primary site of action of analgesic opioids is the central nervous system (CNS). Conventional narcotic analgesics are normally quite hydrophobic and thus are extremely well-suited to permeate lipid membranes, such as the blood-brain barrier. Due to this physical capability, analgesics tend to bind with opioid receptors within the central nervous system in the brain. However, they do not necessarily bind with a homogeneous receptor subtype. This binding causes medically undesirable side effects to occur.

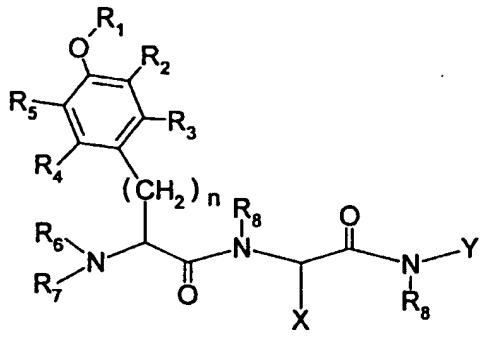
Opiates can cause serious and potentially fatal side effects. Side effects such as respiratory depression, tolerance, physical dependence capacity, and precipitated withdrawal syndrome are caused by nonspecific interactions with central nervous system receptors. See K. Budd, In International Encyclopedia of Pharmacology and Therapeutics; N.E. Williams and H. Wilkinson, Eds., Pergammon: (Oxford), 112, p.51 (1983). Therefore, opioid analgesics acting principally through opioid receptors in the peripheral nervous system would not be expected to cause similar unwanted side effects as those side effects associated with opioid analgesics affecting the central nervous system.

To date, one of the few classes of agents known to exert peripheral analgesic effects are non-steroidal anti-inflammatory agents, such as aspirin, ibuprofen, and ketorolac. These agents do not interact with opioid receptors but are known to inhibit cyclooxygenase and attenuate prostaglandin synthesis. These weak analgesics do not have centrally mediated side effects, but they can cause other side effects such as ulcerations of the gastro-intestinal tract.

There is therefore a need for compounds that exhibit analgesic activity. In particular there is a need for compounds that interact with opioid receptors and more particularly with μ-opioid receptors.

SUMMARY OF THE INVENTION

The present invention provides novel peptidic compounds which act peripherally and are selective for μ-opioid receptors, the compounds represented by formula (I):



(I)

and pharmaceutically acceptable salts thereof wherein

$R_1$ is selected from H, $C_{1-4}$ alkyl and $C_{1-4}$ acyl;

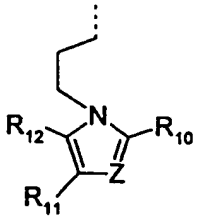
$R_2$ to $R_5$ are independently selected from H, OH, halogen, $C_{1-4}$ alkyl and $C_{1-4}$ alkoxy;

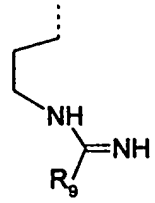
$R_6$ and $R_7$ are independently selected from H and $C_{1-4}$ alkyl;

$R_8$ is H or $C_{1-4}$ alkyl;

n is an integer from 0 to 2;

X is selected from group consisting of (IIa) and (IIb)

IIa 

IIb 

wherein $R_9$ is H, OH, $C_{1-4}$ alkyl, $NH_2$, or $NH-NO_2$; $R_{10}$ to $R_{12}$ are independently H, OH, =O, $NH_2$, $NO_2$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy;

Y is $-CHR_{13}-C(O)-NR_6R_7$, $-CHR_{13}-C(O)-O-R_6$, $-(CHR_{14})_m$-cycloalkyl or $-(CHR_{14})_m$-aryl wherein $R_{13}$ is cycloalkyl, aryl, cycloalkyl-$C_{1-4}$ alkyl or aryl-$C_{1-4}$ alkyl optionally substituted with OH, halogen, $NR_6R_7$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy and $R_{14}$ is H, OH, halogen, $NR_6R_7$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy, and m is an integer from 0 to 5; and

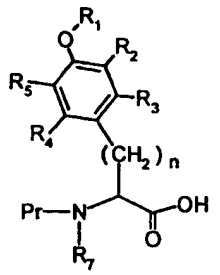
Z is a heteroatom selected from N, O and S.

In another aspect, there is provided pharmaceutical compositions comprising compounds of the present invention and pharmaceutically acceptable carriers, diluents or adjuvants.

In another aspect, there is provided a method of agonizing or activating opioid receptors in a mammal comprising administering to said mammal an opioid receptor agonizing or activating amount of a compound or composition of the invention.

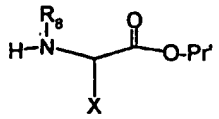
In another aspect, there is provided a method of treating pain in a mammal comprising administering to said mammal an analgesic amount of a compound or composition of the invention.

In another aspect of the invention, there is provided a process for preparing compounds of formula (I) comprising:
coupling a compound of formula (i)



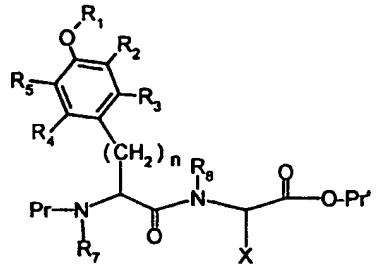

i

wherein $R_1$ to $R_5$, $R_7$ and n are as previously defined and Pr is an amino-protecting group, with a compound of formula (ii)



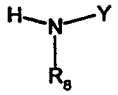
ii

wherein $R_8$ and X are as previously defined and Pr' is a carboxyl-protecting group, to give intermediate of formula (iv)



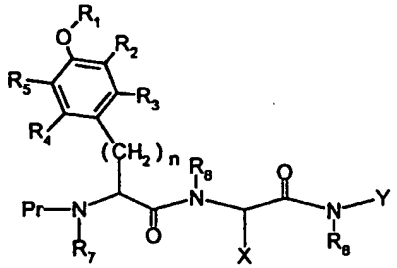
iv ;

removing carboxyl-protecting group Pr' and then coupling intermediate (iv) with a compound of formula (iii)



iii

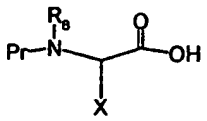
wherein Y is as previously defined, to give an intermediate of formula (v)



v

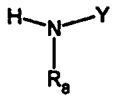
In yet another aspect of the invention, there is provided a process for preparing compounds of formula (I) comprising:

coupling a compound of formula (ii')



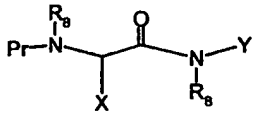
ii'

wherein $R_8$ and X are as previously defined and Pr is an amino-protecting group, with a compound of formula (iii)



iii

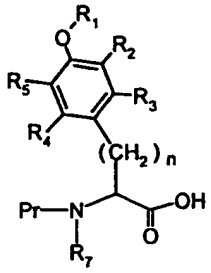
wherein Y is as previously defined, to give intermediate of formula (iv')



iv'

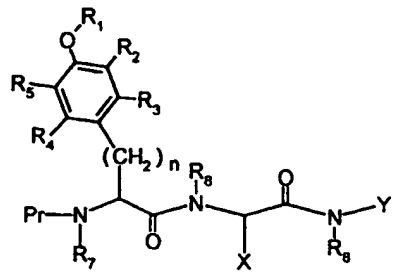
;

removing amino-protecting group Pr and then coupling intermediate (iv') with a compound of formula (i)



i

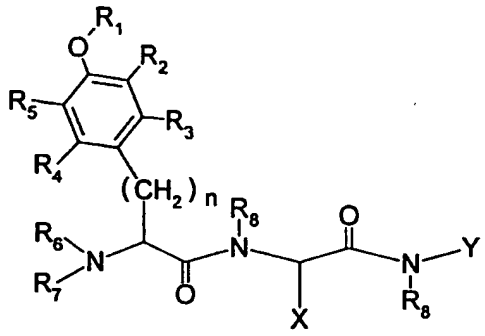
wherein $R_1$ to $R_5$, $R_7$ and n are as previously defined, to give an intermediate of formula (v)



V

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides opioid receptor binding compounds of formula (I)

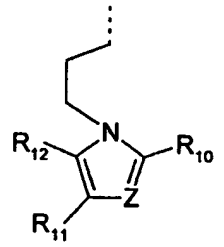


(I)

wherein $R_1$ to $R_8$, X and Y are as defined above.

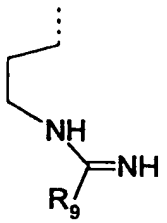
As used herein, the terms "alkyl", "alkoxy" and "acyl" represents straight chain, branched chain, or cyclic hydrocarbon moieties, which are optionally substituted by one or more halogen, hydroxyl or amino ($NR_6R_7$) groups. When used specifically, the term "cycloalkyl" refers to a cyclic alkyl group of 4 to 8 members optionally containing unsaturated bonds and/or heteroatoms N, O or S. Preferred cycloalkyl groups include cyclopentyl, cyclohexyl, and cycloheptyl and is most preferably cyclohexyl. The term "aryl" as used herein represents a 6 to 12 member aromatic carbocycle such as phenyl and naphthyl or a heterocycle such as pyran, pyridine, quinoline, isoquinoline, indole, benzopyran or benzothiazole.

In an embodiment, X is the group (IIa)

IIa 

wherein $R_{10}$ to $R_{12}$ are as previously defined. Z may be O or S thereby forming an oxazole or thiazole ring and is preferably N forming an imidazole ring. Substituents $R_{10}$ to $R_{12}$ are preferably independently H, OH, =O, $NH_2$, $NO_2$ or $C_{1-4}$ alkoxy such as methoxy or ethoxy. It will be appreciated that when any one of $R_{10}$, $R_{11}$ and $R_{12}$ is an oxo group (=O), proper valency of the carbon atom from which the group depends will be maintained i.e. the relevant ring bonds will be single bonds. In a particularly preferred embodiment $R_{10}$ is H, $NH_2$ or $NO_2$ while $R_{11}$ and $R_{12}$ are both H and in a most preferred embodiment each of $R_{10}$ to $R_{12}$ are H.

In another embodiment X is the group (IIb)

IIb 

wherein $R_9$ is H, OH, $C_{1-4}$ alkyl, $NH_2$ or NH-$NO_2$. Preferably $R_9$ is $NH_2$ or NH-$NO_2$ and most preferably $NH_2$.

The group Y is selected from $-CHR_{13}-C(O)-NR_6R_7$, $-CHR_{13}-C(O)-O-R_6$, $-(CHR_{14})_m$-cycloalkyl and $-(CHR_{14})_m$-aryl wherein $R_{13}$ is cycloalkyl, aryl, cycloalkyl-$C_{1-4}$ alkyl or aryl-$C_{1-4}$ alkyl optionally substituted with OH, halogen, $NR_6R_7$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy; $R_{14}$ is H, OH, halogen, $NR_6R_7$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy; and

m is an integer from 0 to 5. In preferred embodiments $R_{13}$ is $C_{1-4}$ alkyl i.e. a methylene group substituted with an optionally substituted aryl or cycloalkyl group. Preferably said aryl group is optionally substituted phenyl, naphthyl, pyridinyl or quinolinyl and said cycloalkyl group is optionally substituted cyclohexyl. Preferred aryl substituents are OH, halogen or $C_{1-4}$ alkyl. More preferably said aryl group is phenyl optionally substituted with halogen and most preferably phenyl optionally substituted at the para-position with fluorine (F).

When Y is $-(CHR_{14})_m$-aryl or $-(CHR_{14})_m$-cycloalkyl, m is preferably 1-5, $R_{14}$ is H, OH, halogen, $C_{1-4}$ alkyl or $NR_6R_7$ and aryl and cycloalkyl are as previously defined. More preferably, m is 3-5 and no more than one $R_{14}$ is OH or $NH_2$ and most preferably, m is 3, no more than one $R_{14}$ is OH, and aryl is a phenyl group and cycloalkyl is cyclohexyl.

$R_1$ is H, $C_{1-4}$ alkyl or $C_{1-4}$ acyl. Preferably $R_1$ is H, methyl or acetyl and most preferably H.

$R_2$ to $R_5$ are independently selected from H, OH, halogen, $C_{1-4}$ alkyl, and $C_{1-4}$ alkoxy. Preferably $R_2$ to $R_5$ are independently H, OH, methyl or methoxy. More preferably $R_2$ and $R_5$ are both H while $R_3$ and $R_4$ are both H or methyl, and most preferably $R_2$ and $R_5$ are H while $R_3$ and $R_4$ are both methyl.

$R_6$ and $R_7$ are independently selected from H and $C_{1-4}$ alkyl. Preferably $R_6$ and $R_7$ are independently H, methyl or ethyl and most preferably are both H.

$R_8$ is H or $C_{1-4}$ alkyl. Preferably $R_8$ is H or methyl and most preferably H.

n is an integer from 0 to 2. Preferably n is 1 or 2 and most preferably 1.

The compounds of the present invention are preferably selected from the group consisting of:

2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (1S-carbamoyl-2-phenyl-ethyl)-amide(compound 1);

2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (1S-carbamoyl-2-phenyl-ethyl)-amide (compound 2);

2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (3-phenylpropyl)-amide, (compound 3, and its diastereomers compound 3a fast), and compound 3b (slow));

2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-(2-nitroimidazol-1-yl)-pentanoic acid (3-phenylpropyl)-amide (compound 4);

2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-(2-amino-imidazol-1-yl)-pentanoic acid (3-phenylpropyl)-amide (compound 5);

2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino],-5-guanidino-pentanoic acid (3-phenyl-propyl)amide (compound 6 and its diastereomers compound 6a (fast) and compound 6b (slow));

2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino],-5-guanidino-pentanoic acid (2-hydroxy-3-phenyl-propyl)amide (compound 7);

H-Tyr-[D]Arg-Phe-$NH_2$ (compound 8);

dimethyltyrosine-[D]ARG-PHE-$NH_2$ (compound 9);

2-{2-[2-aminomethyl-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoylamino}-4-phenyl-butyric acid (compound 10);

N1-[(1S)-1-Carbamoyl-2-phenylethyl]-(2R)-5-(2-amino)-1H-1,3-diazol-1-yl)-2-[(1S0-1-amino-2-(4-hydroxy-2,6-dimethylphenyl)ethylcarboxamide)pentanamide; (compound 11)

(2R)-[(2S)-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoic acid ((1S)-benzyl-2-hydroxy-ethyl)-amide, bistrifluoroacetic acid salts (compound 12);

2S-{2R-[2S-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoylamino}-3-phenyl-propionic acid, bistrifluoroacetic salts (compound 13);

2S-{3-[2R-Amino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl) propionylamino]-2R-methyl-propionylamino}-3-phenyl-propionic acid, trifluoroacetic acid salt(compound 14)

2S-{2R-[2S-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoylamino}-3-phenyl-propionic acid, trifluroroacetic acid salt (compound 15)

Me-Tyr-D-Arg-Phe-OH (compound 16)

(S)-DMT-(OH)-D-Arg--D-Homophe-$OCH_3$(compound 17)
(S)-DMT--D-Arg--D-Homophe-OEt (compound 18)

(S)-DMT-(OH)-D-Arg--L-Homophe-$OCH_3$ (compound 19)
2-{2-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoylamino}-4-phenyl-butyric acid.(compound 20)

and pharmaceutically acceptable salts and derivatives thereof.

The compounds of the present invention are more preferably selected from the group consisting of:

2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (1S-carbamoyl-2-phenyl-ethyl)-amide (compound 1);

2R-[2-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (1S-carbamoyl-2-phenyl-ethyl)-amide (compound 2);

2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (3-phenylpropyl)-amide, (compound 3, and its diastereomers compound 3a fast), and compound 3b (slow));

2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-(2-nitroimidazol-1-yl)-pentanoic acid (3-phenylpropyl)-amide (compound 4);

2-{2-[2-aminomethyl-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoylamino}-4-phenyl-butyric acid (compound 10);

N1-[(1S)-1-Carbamoyl-2-phenylethyl]-(2R)-5-(2-amino)-1H-1,3-diazol-1-yl)-2-[(1S0-1-amino-2-(4-hydroxy-2,6-dimethylphenyl)ethylcarboxamide)pentanamide; (compound 11)

(2R)-[(2S)-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoic acid ((1S)-benzyl-2-hydroxy-ethyl)-amide, bistrifluoroacetic acid salts (compound 12);

2S-{2R-[2S-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoylamino}-3-phenyl-propionic acid, bistrifluoroacetic salts (compound 13);

2S-{2R-[2S-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoylamino}-3-phenyl-propionic acid, trifluroroacetic acid salt (compound 15)

Me-Tyr-D-Arg-Phe-OH (compound 16)

(S)-DMT-(OH)-D-Arg--L-Homophe-$OCH_3$ (compound 19)

2-{2-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoylamino}-4-phenyl-butyric acid. (compound 20)

and pharmaceutically acceptable salts and derivatives thereof.

There is also provided a pharmaceutically acceptable compositions comprising compounds of the present invention and derivatives thereof, in combination with pharmaceutically acceptable carriers diluents or adjuvants. By "derivative" is meant any pharmaceutically acceptable salt, ester, or salt of such ester, of compounds of formula (I) or any other compound which, upon administration to the recipient, is capable of providing (directly or indirectly) compounds of formula (I) or an active metabolite or residue thereof.

It will be appreciated by those skilled in the art that compounds of formula (I), depending on the substituents, may contain one or more chiral centers and thus exist in the form of different isomers, optical isomers (i.e. (+) or (-) enantiomers) and mixtures thereof including racemic mixtures. All such isomers, enantiomers and mixtures thereof including racemic mixtures are included within the scope of the invention. In particular, the chiral carbon atom from which substituent X

depends may have an R or S configuration. Similarly, the chiral carbon atom from which the phenyl group substituted with substituents $R_1$ to $R_5$ depends may be in either R or S configuration as well as any chiral carbon atom that may be incorporated in substituent Y.

Compounds of the present invention may be prepared according to established synthetic techniques such as solution phase or solid phase peptide synthesis from reagents that are commercially available or are prepared according to established synthetic techniques from commercially available reagents. Solid phase synthesis involves the stepwise addition of amino acid residues (of either D or L configuration) to a growing peptide chain that is linked to an insoluble (solid) support or matrix, such as polystyrene. The C-terminal residue of the targeting peptide is first anchored to a commercially available support with its amino group protected with an N-protecting agent such as a fluorenylmethoxycarbonyl (FMOC) group. Typically, the support is obtained with the C-terminal residue preloaded in protected form. The amino protecting group is removed with suitable deprotecting agents such as piperidine and the next amino acid residue (in N-protected form) is added with a coupling agent such as dicyclocarbodiimide (DCC). Upon formation of a peptide bond, the reagents are washed from the support with a suitable reagent such as trifluoroacetic acid (TFA).

Solution phase synthesis of compounds of the invention may be achieved by coupling individual amino acids or derivatives thereof in the following stepwise manner.

a) Intermediates (i) and (ii) are coupled in presence of a suitable activating ester agent such as DCC or EDCI to give intermediate (iv). The N-terminus of (i) is amino-protected with a suitable amino-protecting agent Pr such as Boc, Fmoc or Cbz and the C-terminus of (ii) is protected with a suitable carboxy protecting group Pr' such as benzyl;

# Analgesic Peptidomimetic Compounds

## FIELD OF THE INVENTION

The present invention is related to compounds that exhibit analgesic activity and in particular compounds exhibiting analgesia due to their opioid receptor affinity.

## BACKGROUND OF THE INVENTION

Many endogenous peptides of mammalian and amphibian origin bind to specific opioid receptors and elicit an analgesic response similar to classic narcotic opiates. Many different types of opioid receptors have been shown to coexist in higher animals. For example, see W. Martin *et al.*, J. Pharmacol. Exp. Ther., 197, p. 517(1975); and J. Lord *et al.*, Nature(London), 257, p. 495(1977). Three different types of opioid receptors have been identified. The first, μ, shows a differentiating affinity for morphine and other poly-cyclic alkaloids. The second, δ, shows enhanced selectivity for enkephalin-like peptides. The third, κ, exhibits equal affinity for either group of the above ligands and preferential affinity for dynorphin. In general, the μ-receptors seem to be more involved with analgesic effects. The δ -receptors appear to deal with behavioral effects, although the δ and the κ-receptors may also mediate analgesia.

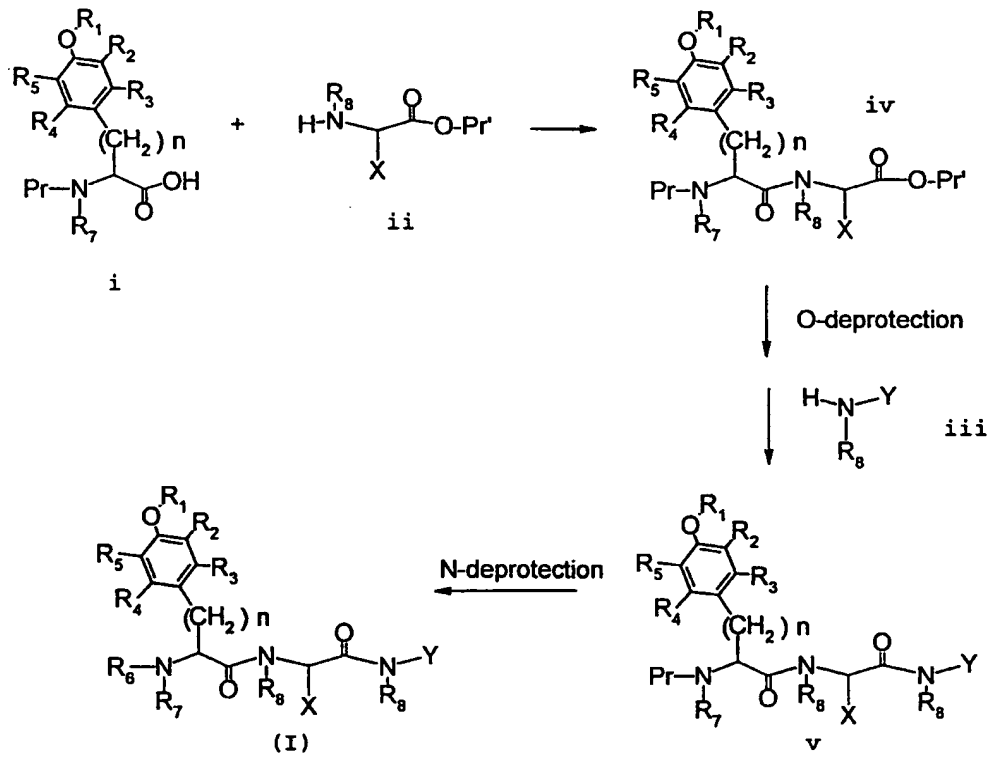
Each opioid receptor, when coupled with an opiate, causes a specific biological response unique to that type of receptor. When an opiate activates more than one receptor, the biological response for each receptor is affected, thereby producing side effects. The less specific and selective an opiate may be, the greater the chance of causing increased side effects by the administration of the opiate.

b) the carboxyl or protecting group Pr' of (iv) is removed with a suitable reagent, for example palladium $H_2$ catalyst when Pr' is benzyl;

c) the carboxyl-deprotected intermediate (iv) is coupled with (iii) in the presence of a suitable ester activating agent to give intermediate (v);

d) Intermediate (v) is amino-deprotected with a suitable deprotecting agent such as TFA when Pr is Boc, piperidine when Pr is Fmoc, or palladium hydrogenation when Pr is Cbz, to give final compound (I).

Scheme I




In an alternative method, compound of formula (I) is prepared by stepwise addition of amino acid derivatives in the reverse order of scheme I as illustrated in scheme II.

a) Intermediates (ii') and (iii') are coupled in the presence of a suitable activating ester agent to give intermediate (iv').

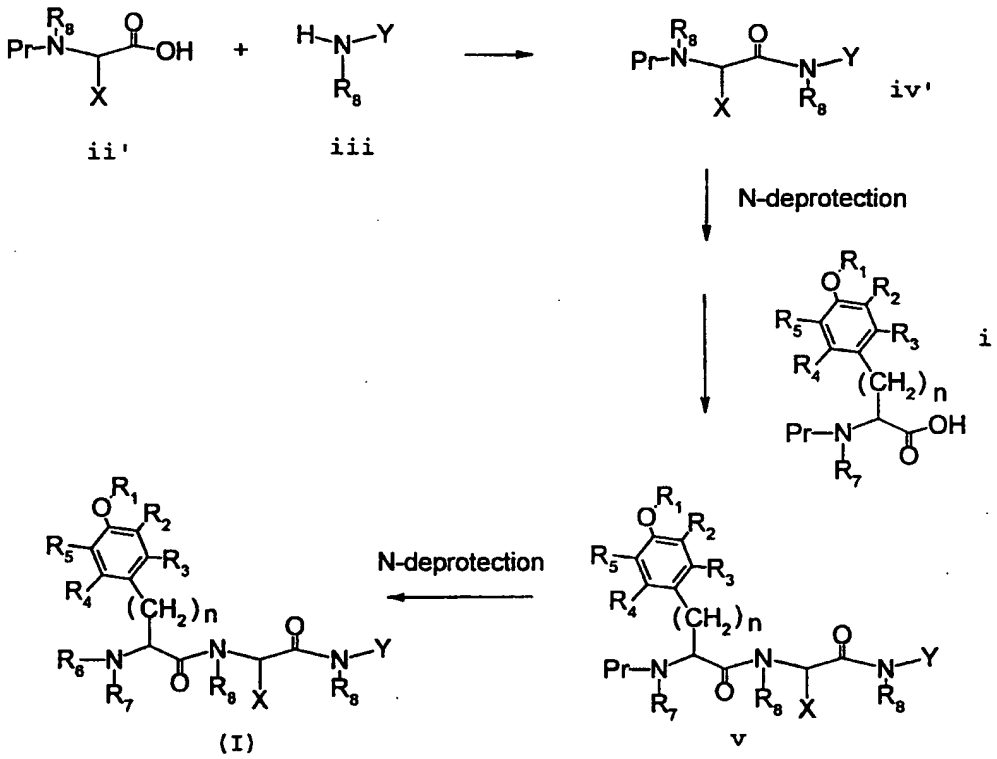
The N-terminus of (ii') is protected with a suitable amino protecting agent Pr;

b) the amino protecting group Pr of (iv') is removed with a suitable reagent;

c) The amino-deprotected intermediate (iv') is coupled with (iii) in the presence of a suitable ester activating agent to give intermediate (v);

d) intermediate (v) is amino-deprotected to give final compound (I).

Scheme II




It is appreciated that depending on the substituents present, that additional protection and deprotection procedures may be necessary at various stages of the above processes illustrated in schemes I and II. Suitable protecting groups i.e. amino, carboxyl or hydroxyl protecting groups, are well known in the field of peptide synthesis and are described in detail in T.W. Greene, Protective Groups In Organic Synthesis, (John Wiley & Sons, 2$^{e}$ edition 1991). The appropriate protecting group for a

particular synthetic scheme will depend on many factors, including the presence of other reactive functional groups and the reaction conditions desired for removal are well known by persons skilled in the art of peptide chemistry.

The present invention also provides pharmaceutical compositions which comprise a pharmaceutically effective amount of a compound of the invention, or pharmaceutically acceptable salts thereof, and preferably, a pharmaceutically acceptable carrier, diluent or adjuvant. The term "pharmaceutically effective amount" is the amount of compound required upon administration to a mammal in order to induce analgesia. Also, the term "opioid receptor agonizing amount" refers to the amount of compound administered to a mammal necessary to bind and/or activate opioid receptors in vivo.

Therapeutic methods of this invention comprise the step of treating patients in a pharmaceutically acceptable manner with those compounds or compositions. Such compositions may be in the form of tablets, capsules, caplets, powders, granules, lozenges, suppositories, reconstitutable powders, or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

In order to obtain consistency of administration, it is preferred that a composition of the invention is in the form of a unit dose. The unit dose presentation forms for oral administration may be tablets and capsules and may contain conventional excipients. For example, binding agents, such as acacia, gelatin, sorbitol, or polyvinylpyrolidone; fillers, such as lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants such as magnesium stearate; disintegrants, such as starch, polyvinylpyrrolidone, sodium starch glycollate or microcrystalline cellulose; or

pharmaceutically acceptable wetting agents such as sodium lauryl sulphate.

The compounds may be administered orally in the form of tablets, capsules, or granules containing suitable excipients such as starch, lactose, white sugar and the like. The compounds may be administered orally in the form of solutions which may contain coloring and/or flavoring agents. The compounds may also be administered sublingually in the form of tracheas or lozenges in which each active ingredient is mixed with sugar or corn syrups, flavoring agents and dyes, and then dehydrated sufficiently to make the mixture suitable for pressing into solid form.

The solid oral compositions may be prepared by conventional methods of blending, filling, tableting, or the like. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of fillers. Such operations are, of course, conventional in the art. The tablets may be coated according to methods well known in normal pharmaceutical practice, in particular with an enteric coating.

Liquid oral preparations may be in the form of emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may or may not contain conventional additives. For example suspending agents, such as sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminum stearate gel, or hydrogenated edible fats; emulsifying agents, such as sorbitan monooleate or acaci; non-aqueous vehicles (which may include edible oils), such as almond oil, fractionated coconut oil, oily esters selected from the group consisting of glycerine, propylene glycol, ethylene glycol, and ethyl alcohol; preservatives, for instance methyl para-hydroxybenzoate, ethyl para-

hydroxybenzoate, n-propyl parahydroxybenzoate, or n-butyl parahydroxybenzoate of sorbic acid; and, if desired, conventional flavoring or coloring agents.

The compounds may be injected parenterally; this being intramuscularly, intravenously, or subcutaneously. For parenteral administration, the compound may be used in the form of sterile solutions containing other solutes, for example, sufficient saline or glucose to make the solution isotonic. For parenteral administration, fluid unit dosage forms may be prepared by utilizing the compound and a sterile vehicle, and, depending on the concentration employed, may be either suspended or dissolved in the vehicle. Once in solution, the compound may be injected and filter sterilized before filling a suitable vial or ampoule and subsequently sealing the carrier or storage package. Adjuvants, such as a local anesthetic, a preservative or a buffering agent, may be dissolved in the vehicle prior to use. Stability of the pharmaceutical composition may be enhanced by freezing the composition after filling the vial and removing the water under vacuum, (e.g., freeze drying the composition). Parenteral suspensions may be prepared in substantially the same manner, except that the compound should be suspended in the vehicle rather than being dissolved, and, further, sterilization is not achievable by filtration. The compound may be sterilized, however, by exposing it to ethylene oxide before suspending it in the sterile vehicle. A surfactant or wetting solution may be advantageously included in the composition to facilitate uniform distribution of the compound.

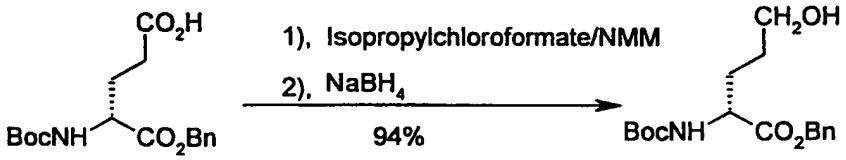
The pharmaceutical compositions of this invention comprise a pharmaceutically effective amount of a compound of this invention and a pharmaceutically acceptable carrier. Typically, they contain from about 0.01% to about 99% by weight, preferably from about 10% to about 60% by weight, of a compound of this

invention, depending on which method of administration is employed.

In another aspect of the invention, compounds may be used to identify opioid receptors from non-opioid receptors. For such use, compounds of the invention are radiolabeled e.g. by incorporating $^{3}H$ or $^{14}C$ within its structure or by conjugation to $^{125}I$. Such radiolabeled forms can be used directly to identify the presence of opioid receptors and in particular $\mu$ opioid receptors in a receptor population. This can be achieved by incubating membrane preparations with a radiolabeled compound of the invention. The presence and or amount of opioid receptors in the preparation is determined from the difference in membrane-bound radioactivity against a control preparation devoid of opioid receptors. Furthermore, radiolabeled forms of the present compounds can be exploited to screen for more potent opioid ligands, by determining the ability of the test ligand to displace the radiolabeled compound of the present invention.

EXAMPLE 1 Preparation of intermediate 2R-amino-5-imidazol-1-yl-pentanoic acid benzyl ester

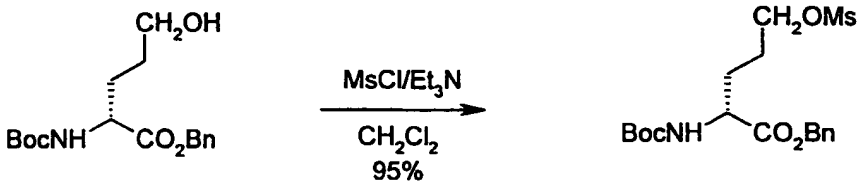
Step 1 2R-tert-Butoxycarbonylamino-5-hydroxy-pentanoic acid benzyl ester




Isopropylchloroformate (1 M in toluene, 2.67 mmol, 2.67 ml) was added to a mixture solution of NMM and Boc-R-Glu-OBn (1.00 g, 2.96 mmol) in THF (70 ml) at -15°C. The resulting solution was stirred for 1 hr and was added to a solution of $NaBH_4$ in dry THF/MeOH (1:4) (100 ml) at -78°C via annulation. The reaction mixture was stirred at -78°C for 3 hr. Acetic acid (2.5 ml) was

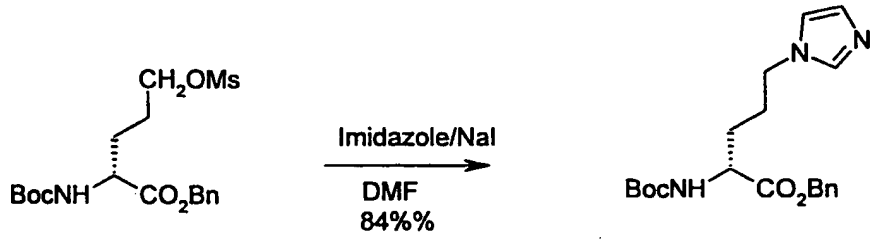
added and then warmed to ambient temperature. Solvent was evaporated and the residue dissolved in ethylacetate, washed with saturated $NaHCO_3$ aqueous solution and brine, dried over $MgSO_4$, and then filtered. The filtrate was evaporated to give the desired product as oil (0.900 g, 94%).

Step 2 2R-tert-butoxycarbonylamino-5-methanesulfonyloxy-pentanoic acid benzyl ester



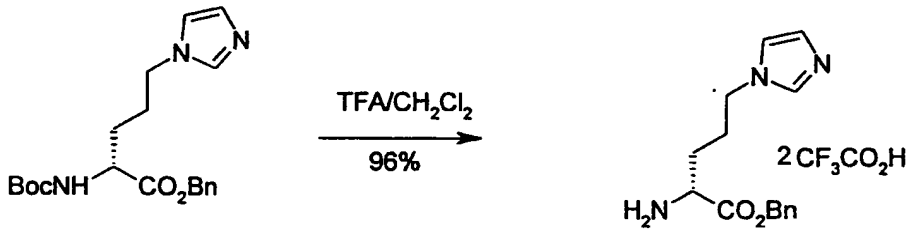

Methanesulfonyl chloride (0.378 g, 0.255 ml, 3.30 mmol) was added to a solution of 2R-tert-butoxycarbonylamino-5-hydroxy-pentanoic acid benzyl ester (0.900 g, 0.278 mmol) and triethylamine (0.455 g, 0.640 ml, 4.50 mmol) in dichloromethane (50 ml) at 0°C. The mixture was stirred for 30 min. then poured into ice water and extracted with dichloromethane (50 ml) three times. The combined solution was dried over $MgSO_4$, filtered, then evaporated to give the desired product as an oil (1.06 g, 95%).

Step 3 2R-tert-butoxycarbonylamino-5-imidazol-1-yl-pentanoic acid benzyl ester



A mixture of imidazole (1.00 g, 15.0 mmol), NaI (0.450 g, 3.00 mmol) and 2R-tert-butoxycarbonylamino-5-methanesulfonyloxy-pentanoic acid benzyl ester (1.06 g, 0.264 mmol) in dry DMF (10 ml) was stirred at 80°C for 3 hr. and then cooled to ambient temperature. The DMF was evaporated and the residue was partitioned between ethylacetate and saturated $NaHCO_3$ aqueous solution. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated to yield the desired product as oil (0.830 g, 84%).

Step 4 2R-amino-5-imidazol-1-yl-pentanoic acid benzyl ester

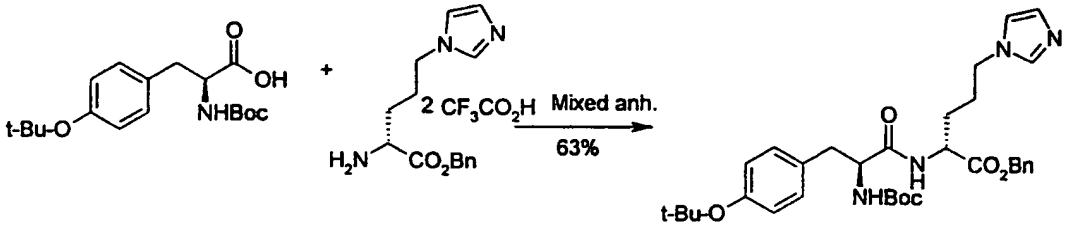



2R-tert-butoxycarbonylamino-5-imidazol-1-yl-pentanoic acid benzyl ester (0.830 g, 2.22 mmol) was dissolved in TFA/$CH_2Cl_2$ (1:1, 10 ml) at ambient temperature and stirred for 1 hr. Solvent was then evaporated to give the desired product as an oil (1.07 g, 96%).

$^1H$ NMR (acetone -$d_6$) δ: 2.25 (m, 4H), 4.50 (m, 2H), 5.24 (m, 3H), 7.35 (m, 5H), 7.69 (s, 1H), 7.75 (s, 1H), 7.76 (s, 1H), 9.07 (br, 2H).

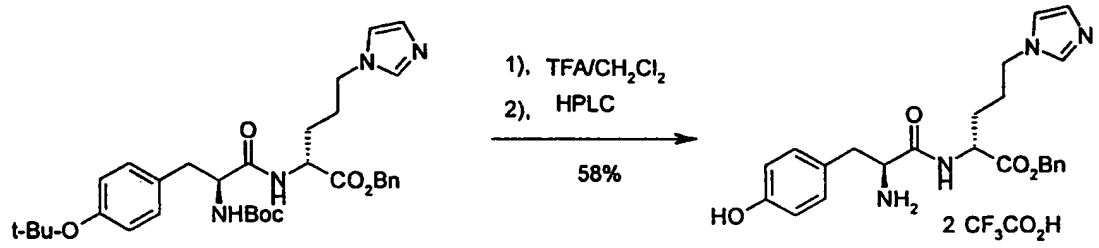
EXAMPLE 2 Preparation of 2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (1S-carbamoyl-2-phenyl-ethyl)-amide (compound 1)

Step 1 2R-[2S-tert-butoxycarbonylamino-3-(4-tert-butoxy-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoic acid benzyl ester




Boc-S-Tyr(t-Bu)-OH (0.840 g, 2.22 mmol) in dichloromethane (20 ml) was cooled to 0°C ($N_2$ atmosphere), and triethylamine (0.337 g, 0.464 ml, 3.33 mmol) was added, then isobutyl chloroformate (0.273 g, 0.260 ml, 2.00 mmol) was added. The reaction mixture was stirred for 1 hr. 2-R-amino-5-imidazol-1-yl-pentanoic acid benzyl ester (1.07 g, 2.15 mmol) was added. The reaction mixture was warmed to room temperature and stirred for another hour. The mixture was then diluted with dichloromethane, washed with 10% aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$, filtered, and concentrated. The residue was chromatographed on silica gel using ethyl acetate / methanol (9:1) as eluant to provide the desired product as white solid (0.631 g, 63%).

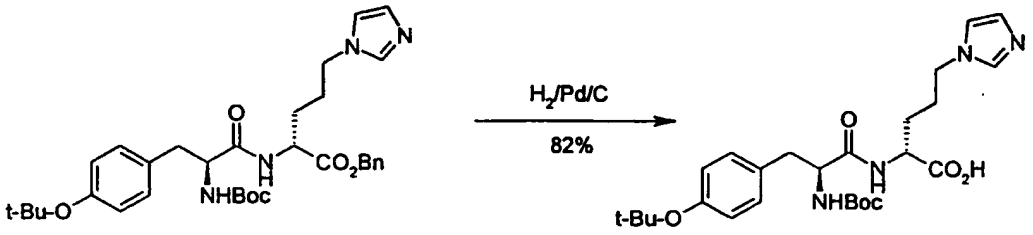
Step 2 2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid benzyl ester




2R-[2S-tert-butoxycarbonylamino-3-(4-tert-butoxy-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid benzyl ester(0.230 g, 0.388 mmol) was dissolved in TFA/$CH_2Cl_2$ (1:1, 10

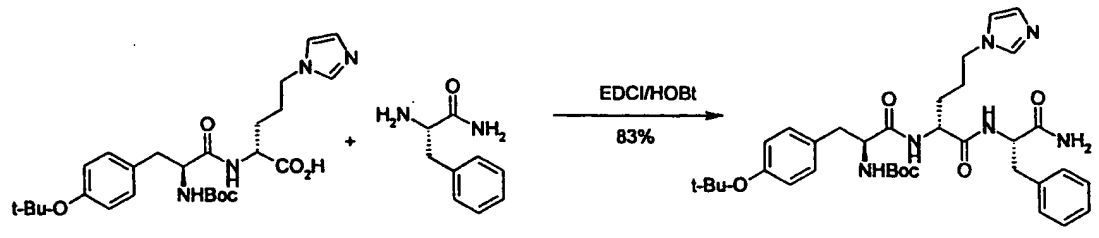
ml) at ambient temperature and stirred for 1 hr. Solvent was then evaporated and the residue purified by HPLC (C-18) using a 20- 50% acetonitrile (0.1% (v/v) TFA)/aqueous( 0.1% (v/v) TFA) gradient elution to give the desired product as white powder (0.160 g, 58%).

Step 3 2R-[2S-tert-butoxycarbonylamino-3-(4-tert-butoxy-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoic acid



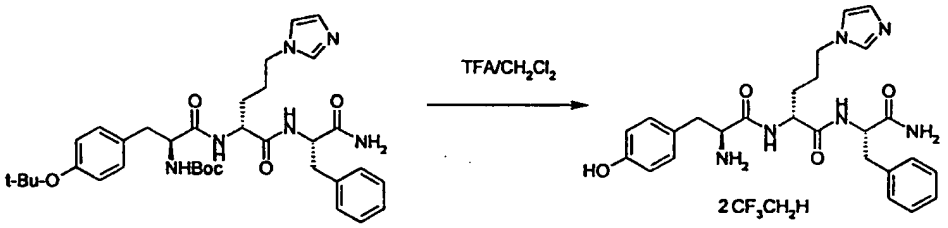

2R-[2S-tert-butoxycarbonylamino-3-(4-tert-butoxy-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid benzyl ester(0.400 g, 0.675 mmol) was dissolved in methanol (10 ml) and Pd/C (0.0720 g, 10%) was added. The resulting mixture was stirred under hydrogen at ambient temperature and stirred for 0.5 hr. Catalyst was filtered off. The filtrate was evaporated to give the desired product as white powder (0.279 g, 82%).

Step 4 {2-(4-tert-butoxy-phenyl)-1S-[1R-(1S-carbamoyl-2-phenyl-ethylcarbamoyl)-4-imidazol-1-yl-butylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester



To a mixture of 2R-[2S-tert-butoxycarbonylamino-3-(4-tert-butoxy-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoic acid (0.279 g, 0.555 mmol), HOBt (0.127 g, 0.941 mmol) and S-phenylalaninamide (0.0912 g, 0.555 mmol) in DMF (10 ml) was added EDCI (0.160 g, 0.833 mmol) at 0°C. Then it was warmed to ambient temperature and stirred overnight. DMF was evaporated. The residue was partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give the desired product as white solid (0.301 g, 83%).

Step 5 2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (1S-carbamoyl-2-phenyl-ethyl)-amide (BCH 6377)




(1)

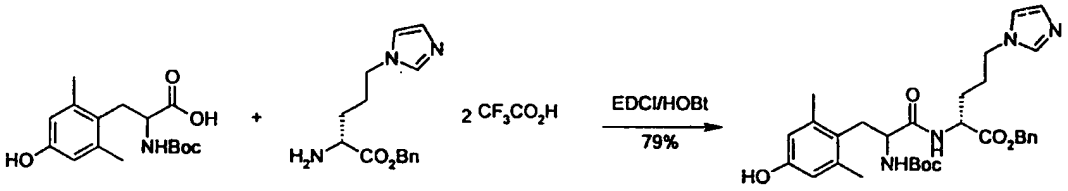
{2-(4-tert-Butoxy-phenyl)-1S-[1R-(1S-carbamoyl-2-phenyl-ethylcarbamoyl)-4-imidazol-1-yl-butylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester (0.301 g, 0.464 mmol) was dissolved in $TFA/CH_2Cl_2$ (1:1, 10 ml) at ambient temperature and stirred for 1 hr. Solvent was evaporated. The residue was purified by HPLC (C-18) using a 20- 50% acetonitrile (0.1% (v/v) TFA)/aqueous( 0.1% (v/v) TFA) gradient elution to give the desired product as white powder (0.205 g, 61%).

$^1H$ NMR (DMSO -$d_6$) δ: 1.02-1.20 (m, 4H), 2.65 (m, 1H), 2.80 (m, 2H), 3.05 (m, 1H), 3.95 (m, 3H), 4.35 (m, 1H), 4.48 (m, 1H), 6.67 (d, 2H, J=8.4 Hz), 7.00 (d, 2H, J=8.4 Hz), 7.15 (m, 5 H), 7.54 (s, 1H), 7.59 (s, 1H), 7.73 (s, 1H), 8.10 ( br, 3H), 8.45

(d, 1H, J=9.0 Hz), 8.51 (d, 1H, J=9.0 Hz), 9.00 (s, 1H), 9.37 (br, 1H). MS: (m/z) 591.7.

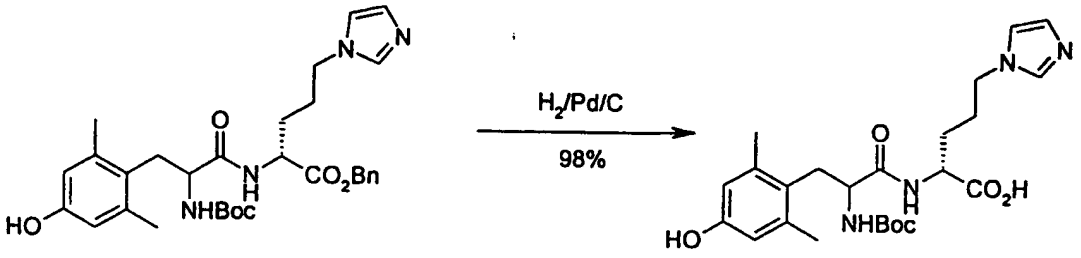
EXAMPLE 3 2R-[2-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (1S-carbamoyl-2-phenyl-ethyl)-amide (compound 2)

Step 1 2R-[2-tert-butoxycarbonylamino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoic acid benzyl ester ( diastereomer mixture)



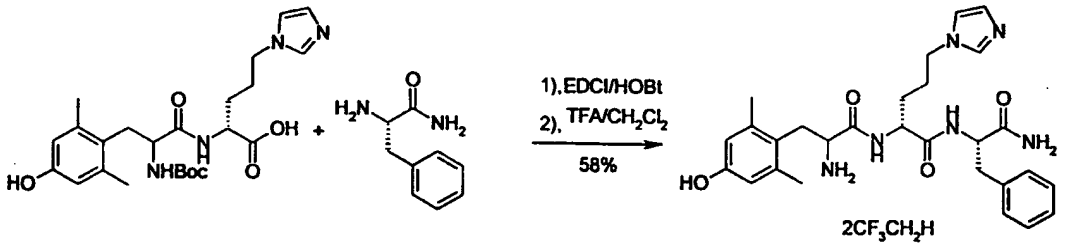

To a mixture solution of 2-R-amino-5-imidazol-1-yl-pentanoic acid benzyl ester (0.812 g, 1.62 mmol), HOBt (0.372 g, 2.76 mmol) and Boc-(RS)2,6-$Me_2$Tyr-OH (0.500 g, 1.62 mmol) in DMF (10 ml) was added EDCI (0.465 g, 2.43 mmol) at 0°C. Then it was warmed to ambient temperature and stirred overnight. DMF was evaporated. The residue was partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give the desired product as white solid (0.717 g, 79%).

Step 2 2R-[2-tert-butoxycarbonylamino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoic acid (diastereomer mixture)




2R-[2-tert-Butoxycarbonylamino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoic acid benzyl ester ( Mixture)(0.700 g, 1.24 mmol) was dissolved in methanol (10 ml) Pd/C (0.0660 g, 10%) was added. The resulting mixture was stirred under hydrogen at ambient temperature for 0.5 hr. Catalyst was filtered off. The filtrate was evaporated to give the desired product as white solid (0.574 g, 98%).

Step 3 2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (1S-carbamoyl-2-phenyl-ethyl)-amide

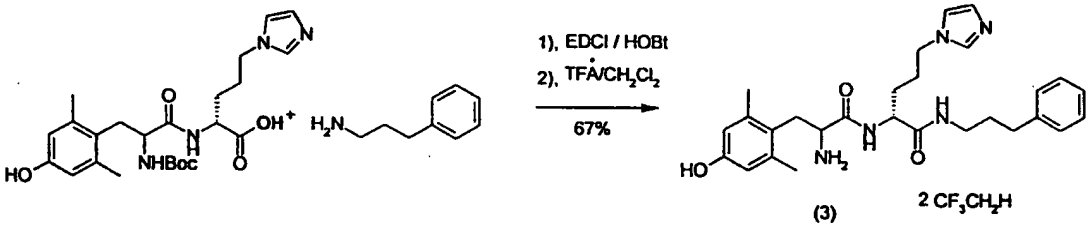



(2)

To a mixture solution of 2R-[2-tert-butoxycarbonylamino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoic acid (mixture) (0.288 g, 0.607 mmol), HOBt (0.139 g, 1.03 mmol) and S-phenylalaninamide (0.0997 g, 0.607 mmol) in DMF (10 ml) was added EDCI (0.150 g, 0.911 mmol) at 0°C. Then it was warmed to ambient temperature and stirred overnight. DMF was evaporated. The residue was partitioned between ethylacetate and

water. The organic phase was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtered. The filtrate was evaporated to give [1-[1R-(1S-carbamoyl-2-phenyl-ethylcarbamoyl)-4-imidazol-1-yl-butylcarbamoyl]-2-(4-hydroxy-2,6-dimethyl-phenyl)-ethyl]-carbamic acid tert-butyl ester (mixture)(0.325 g, 0.524 mmol) which was dissolved in TFA/$CH_2Cl_2$ (1:1, 10 ml) at ambient temperature and stirred for 1 hr. Solvent was evaporated. The residue was purified by HPLC using a gradient A/B (80/20 to 50/50) ( A: 0.1% (v/v) TFA aqueous, B: 0.1% (v/v) acetonitrile), followed by lyophilization of aqueous solution to give the desired product as white powder (0.268 g, overall yield 58%). $^1H$ NMR (methanol -$d_4$) δ: (partial), 2.49 and 2.50 (2s, 9H), 6.35 and 6.36 (2s, 2H). MS: (m/z) 520.1.

EXAMPLE 4 2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (3-phenylpropyl)-amide, (compound 3 diastereomer mixture, compound 3a fast diastereomer, and compound 3b slow diastereomer)



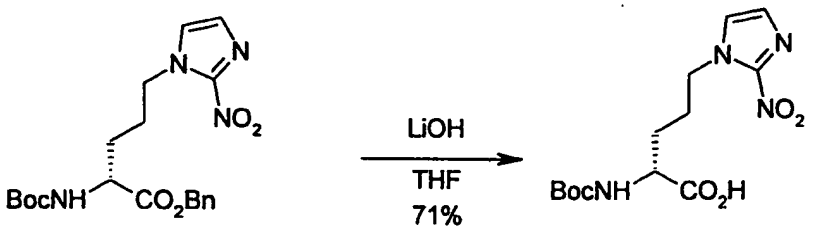

To a mixture solution of 2R-[2-tert-butoxycarbonylamino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoic acid (mixture) (0.254 g, 0.536 mmol), HOBt (0.123 g, 0.911 mmol) and 3-phenylpropylamine (0.0797 g, 0.589 mmol) in DMF (10 ml) was added EDCI (0.154 g, 0.806 mmol) at 0°C then warmed to ambient temperature and stirred overnight. DMF was evaporated and the residue partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$ and

brine, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give {2-(4-Hydroxy-2,6-dimethyl-phenyl)-1R- [4-imidazol-1-yl-1-(3-phenyl-propylcarbamoyl)-butylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester (mixture)(0.272 g, 0.460 mmol) which was dissolved in TFA/$CH_2Cl_2$ (1:1, 10 ml) at ambient temperature and stirred for 1 hr. Solvent was evaporated and the residue purified by HPLC (C-18) using a 20- 50% acetonitrile (0.1% (v/v) TFA)/aqueous( 0.1% (v/v) TFA) gradient elution to give the two product diastereomers as white powder Fast diastereomer(0.150 g) and slow diastereomer (0.108 g). In a HPLC elution system (C-18, 0-30% acetonitrile (0.1% (v/v) TFA)/aqueous( 0.1% (v/v) TFA) gradient elution, diastereomer Fast is the fast moving compound while diastereomer Slow is the slower moving.

$^1H$ NMR (DMSO -$d_6$) δ: Diastereomer Fast(rt=44.70), 1.13-1.30 (m, 4H), 1.59-1.67 (m, 2H), 2.17 (s, 6H), 2.50 (m, 2H), 2.85 (m, 1H), 3.01 (m, 3H), 3.85 (m, 1H), 4.02 (m, 2H), 4.15 (m, 1H), 6.40 (s, 2H), 7.13-7.28 (m, 5H), 8.00 (t, 1H, J=5.6 Hz), 8.31 (d, 1H, J=8.1 Hz), 8.36 (br, 3H), 8.99 (s, 1H), 9.15 (br, 1H). MS: (m/z) 491.1. Diastereomer Slow(rt=52.96), 1.38-1.49 (m, 2H), 1.62-1.71 (m, 4H), 2.12 (s, 6H), 2.56 (t, 2H, J=7.6 Hz), 2.79 (dd, 1H, J=14.0 and 4.2 Hz), 3.01 (m, 3H), 3.83 (br, 1H), 4.16 (t, 2H, J=7.0 Hz), 4.29 (m, 1H), 6.36 (s, 2H), 7.16-7.30 (m, 5H), 7.65 (s, 1H), 7.69 (s, 1H), 7.78 (t, 1H, J=5.6 Hz), 8.25 (d, 1H, J=8.3 Hz), 8.32 (br, 3H), 9.00 (s, 1H), 9.05 (s, 1H). MS: (m/z) 490.8.

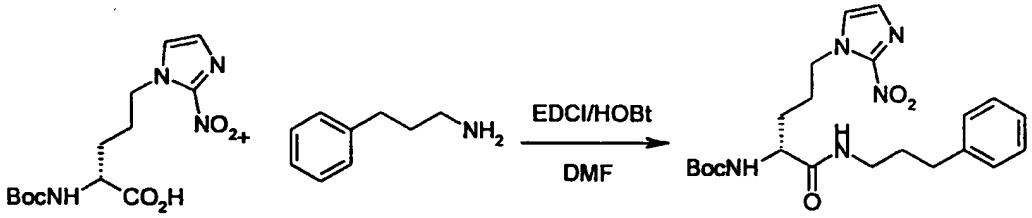
EXAMPLE 5 2R-[2S-Amino-3-(4-hydroxy-phenyl)-propionylamino]-5-(2-nitroimidazol-1-yl)-pentanoic acid (3-phenylpropyl)-amide (compound 4)

Step 1 2-tert-butoxycarbonylamino-5-(2-nitro-imidazol-1-yl)-pentanoic acid




An aqueous solution of $LiOH.H_2O$ (0.125 g, 2.97 mmol) (10 ml) was added to a solution of 2R-tert-butoxycarbonylamino-5-(2-nitro-imidazol-1-yl)-pentanoic acid benzyl ester (0.830 g, 1.98 mmol) in THF (10 ml) at 0°C. The reaction mixture was then warmed to ambient temperature and stirred for 1 hr. The reaction solution was extracted twice with ethylacetate. The aqueous phase was acidified with 5% aqueous solution of $KHSO_4$, extracted with ethylacetate. The organic phase was washed with brine, dried over $MgSO_4$, filtered and evaporated to give the desired product as white solid (0.465 g, 71%).

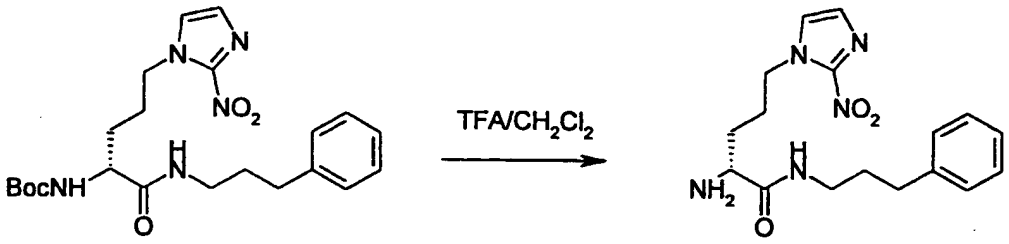
Step 2 [4-(2-nitro-imidazol-1-yl)-1-(3-phenyl-propylcarbamoyl)-butyl]-carbamic acid tert-butyl ester




To a mixture solution of 2R-tert-butoxycarbonylamino-5-(2-nitro-imidazol-1-yl)-pentanoic acid (0.465 g, 1.42 mmol), HOBt (0.325 g, 2.41 mmol) and 3-phenylpropylamine (0.287 g, 2.13 mmol) in DMF (10 ml) was added EDCI (0.407 g, 2.13 mmol) at 0°C. Then it was warmed to ambient temperature and stirred overnight. DMF was evaporated. The residue was partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$ and

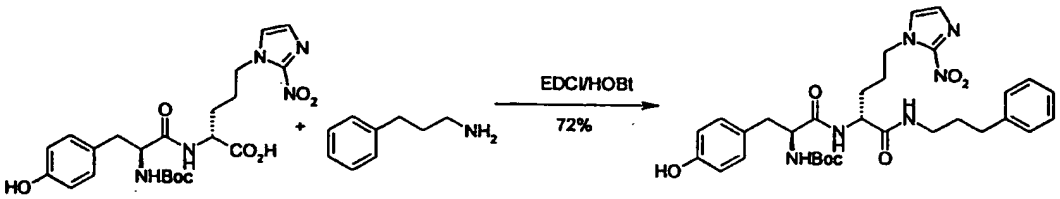
brine, dried over $MgSO_4$, filtered. The filtrate was evaporated to give the desired product as white solid (0.420 g, 67%).

Step 3 2R-amino-5-(2-nitro-imidazol-1-yl)-pentanoic acid (3-phenyl-propyl)-amide




[4-(2-nitro-imidazol-1-yl)-1-(3-phenyl-propylcarbamoyl)-butyl]-carbamic acid tert-butyl ester(0.420 g, 0.943 mmol) was dissolved in TFA/$CH_2Cl_2$ (1:1) (10 ml) and the solution stirred at room temperature for 1 hr. The solvent was evaporated and the residue dissolved in ethyl acetate, washed with sat. $NaHCO_3$ aqueous solution and brine, dried over $Na_2SO_4$, and filtered. The filtrate was then evaporated to yield the desired product as oil (0.320 g, 99%).

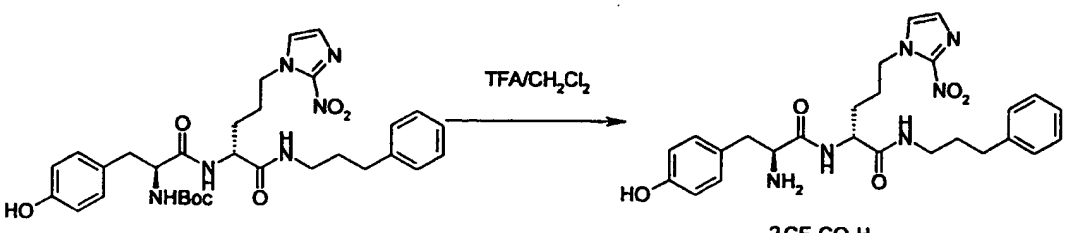
Step 4 {2S-(4-hydroxy-phenyl)-1R- [4-(2-nitro-imidazol-1-yl-1-(3-phenyl-propylcarbamoyl)-butylcarbamoyl]- ethyl}-carbamic acid tert-butyl ester




To a mixture solution of 2R-amino-5-(2-nitro-imidazol-1-yl)-pentanoic acid (3-phenyl-propyl)-amide (0.320 g, 0.927 mmol), HOBt (0.188 g, 1.39 mmol) and Boc-S-Tyr-OH (0.287 g, 0.927 mmol) in DMF (10 ml) was added EDCI (0.267 g, 1.39 mmol) at 0°C. Then

it was warmed to ambient temperature and stirred overnight. DMF was evaporated. The residue was partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtered. The filtrate was evaporated to give the desired product as white solid (0.477 g, 72%).

Step 5 2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-(2-nitroimidazol-1-yl)-pentanoic acid (3-phenylpropyl)-amide

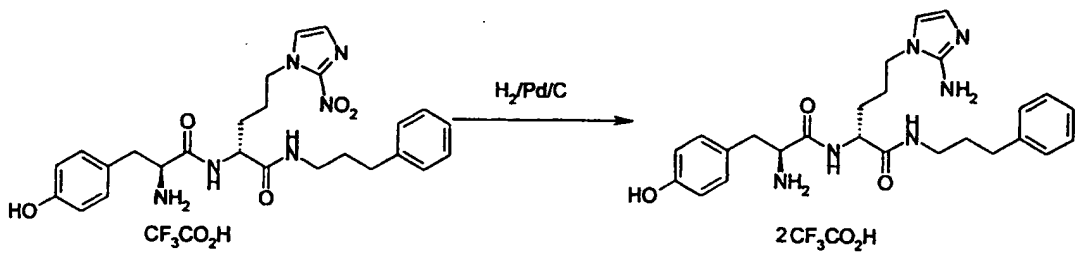



(4)

{2S-(4-hydroxy-phenyl)-1R-[4-(2-nitro-imidazol-1-yl-1-(3-phenyl-propylcarbamoyl)-butylcarbamoyl]- ethyl}-carbamic acid tert-butyl ester (0.477 g, 0.784 mmol) was dissolved in TFA/$CH_2Cl_2$ (1:1, 10 ml) at ambient temperature and stirred for 1 hr. Solvent was evaporated. The residue was purified by HPLC using a gradient A/B (0 to 70/30) ( A: 0.1% (v/v) TFA aqueous, B: 0.1% (v/v) acetonitrile), followed by lyophilization of aqueous solution to give the desired product as white powder (0.443 g, 79%).

$^1$H NMR (DMSO -$d_6$) δ: 1.31-1.69 (m, 6H), 2.53 (t, 2H, J=7.5 Hz), 2.65 (m, 1H), 2.85 (m, 1H), 2.93 (m, 1H), 3.09 (m, 1H), 3.99 (m, 1H), 4.33 (m, 3H), 6.69 (d, 2H, J=8.5 Hz), 7.02 (d, 2H, J=8.5 Hz), 7.14-7.28 (m, 6H), 7.63 (s, 1H), 8.05 (br, 3H), 8.12 (t, 1H, J=5.5 Hz), 8.59 (d, 1H, J=8.2 Hz), 9.36 (br, 1H).

EXAMPLE 6 2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-(2-amino-imidazol-1-yl)-pentanoic acid (3-phenylpropyl)-amide (compound 5)



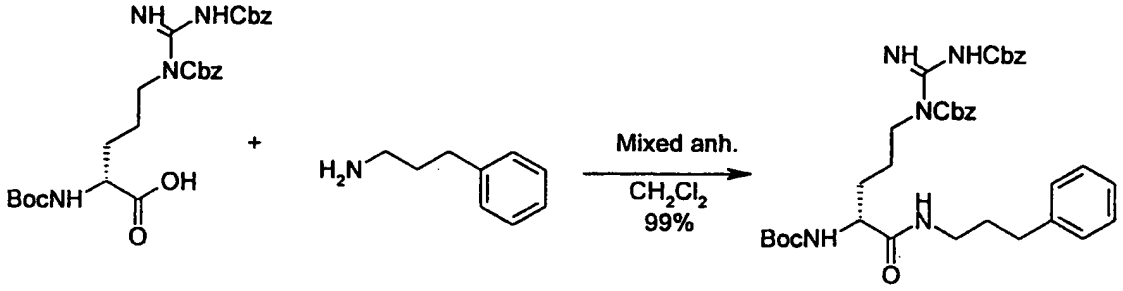

(5)

2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-(2-nitroimidazol-1-yl)-pentanoic acid (3-phenylpropyl)-amide (0.300 g, 0.419 mmol) was dissolved in methanol (10 ml) Pd/C (0.045 g) was added. The solution was stirred under hydrogen for 2 hr. then the catalyst was filtered off and the filtrate evaporated. The residue was purified by HPLC using a gradient A/B (0 to 70/30) ( A: 0.1% (v/v) TFA aqueous, B: 0.1% (v/v) acetonitrile), followed by lyophilization of aqueous solution to give the desired product as white powder (0.250 g, 83%).

$^1$H NMR (DMSO -$d_6$) δ: 1.38-1.51 (m, 4H), 1.68 (m, 2H), 2.54 (t, 2H, J=7.6 Hz), 2.80 (m, 1H), 2.90 (m, 1H), 3.00 (m, 2H), 3.77 (br, m, 2H), 3.97 (t, 1H, J=7.2 Hz), 4.27 (m, 1H), 6.69 (d, 1H, J=8.5 Hz), 6.89 (s, 2H), 7.02 (d, 2H, J=8.5 Hz), 7.17 (m, 5H), 7.54 (br, s, 2H), 8.14 (t, 1H, J=5.6 Hz), 8.58 (d, 1H, J=8.1 Hz), 9.35 (br, 1H). MS: (m/z) 478.1.

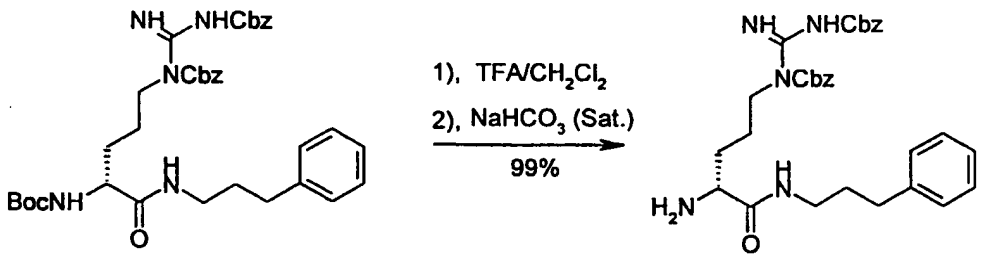
EXAMPLE 7 2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino],-5-guanidino-pentanoic acid (3-phenyl-propyl)amide (compound 6 diastereomer mixture, compound 6a fast diastereomer, compound 6b slow diastereomer)

Step 1 Preparation of Boc-R-Arg($Z_2$)-PPA (PPA=3-phenylpropylamine)



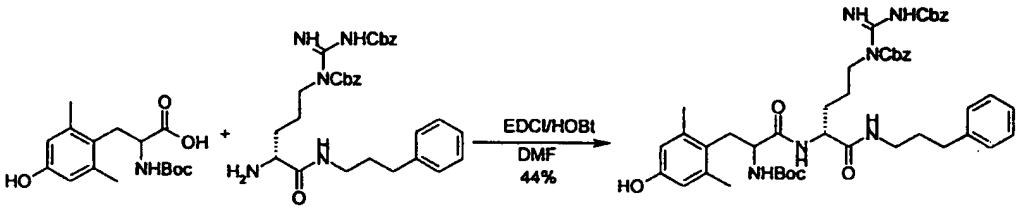

Boc-R-Arg($Z_2$)-OH (0.500 g, 1.01 mmol) in dichloromethane (10 ml) was cooled to 0°C ($N_2$ atmosphere) and triethylamine (0.123 g, 0.169 ml, 1.21 mmol) was added. To this, isobutyl chloroformate (0.137 g, 0.130 ml, 1.01 mmol) was added dropwise and the reaction mixture stirred for 1 hr at this temperature. 3-Phenylpropylamine (0.150 g, 0.158 ml, 1.11 mmol) was added dropwise and this mixture was stirred for 1 hr at and then allowed to warm to room temperature and stirred for another 1 hr. The mixture was diluted with dichloromethane (40 ml), washed with 10% aqueous $KHSO_4$ (30 ml), saturated aqueous $NaHCO_3$ (30 ml), and brine (30 ml). The organic extracts were then dried over $MgSO_4$, and concentrated. The residue was purified by chromatography on silica gel eluted with ethyl acetate / dichloromethane (1:1) to give the desired product as white solid (0.600 g, 99%).

Step 2 R-Arg($Z_2$)-PPA (PPA=3-Phenylpropylamine)



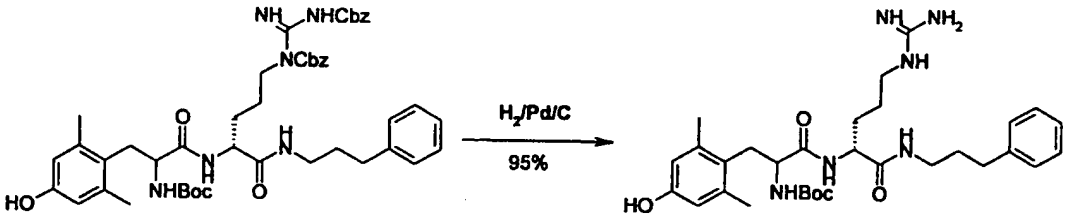
Boc-R-Arg($Z_2$)-PPA (0.600 g, 0.909 mmol) was dissolved in TFA/$CH_2Cl_2$ (1:1) (10 ml). The solution was stirred at room temperature for 1 hr. The solvent was evaporated. The residue was dissolved in ethyl acetate( 30 ml), washed with sat. $NaHCO_3$ aqueous solution (50 ml) and brine(30 ml), dried over $Na_2SO_4$, filtered, and the filtrate was evaporated to yield the desired product as an oil (0.504 g, 99%)

Step 3 Boc-(RS)2,6-$Me_2$Tyr-R-Arg($Z_2$)-PPA



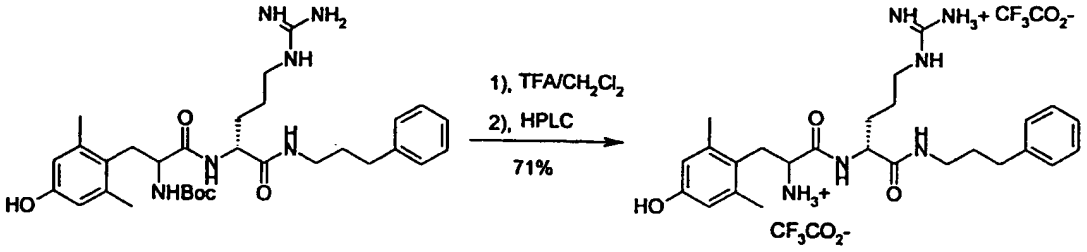

To a solution of Boc-(RS)2,6-$Me_2$Tyr-OH (0.313 g, 1.03 mmol), R-Arg($Z_2$)-PPA (0.504 g, 0.901 mmol) and HOBt.$H_2O$ (0.209 g, 1.55 mmol) in DMF (10 ml) was added EDCI (0.296 g, 1.55 mmol) under nitrogen at 0°C. After stirring overnight at room temperature, the reaction mixture was diluted with ethyl acetate, washed with 10% $KHSO_4$ aqueous solution, saturated $NaHCO_3$ aqueous solution and brine, then dried over $MgSO_4$, filtered, and concentrated. The residue was chromatographed on silica gel using ethyl acetate/dichloromethane (3:7) as eluant to provide the desired product (0.337 g, 44%) as a racemic mixture.

Step 4 Boc-(RS)2,6-$Me_2$Tyr-R-Arg-PPA




Boc-(RS)2,6-$Me_2$Tyr-R-Arg($Z_2$)-PPA (2.48 g, 2.91 mmol) was dissolved in methanol (30 ml). Pd/c (0.31 g) was added. The solution was stirred under hydrogen at room temperature for 1 hr. and the catalyst was filtered off. The filtrate was evaporated to give the desired product as a white solid (1.60 g, 95%).

Step 5 2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoic acid(3-phenyl-propyl)amide (BCH 6019 = diastereomer mixture, BCH6925 = diastereomer fast, BCH6927 = diastereomer slow)



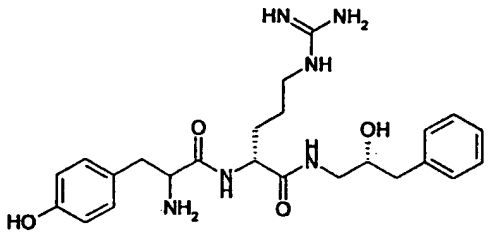

(6)

Boc-(RS)2,6-$Me_2$Tyr-R-Arg-PPA (1.60 g, 2.75 mmol) was dissolved in TFA/$CH_2Cl_2$ (1:1) (10 ml). The solution was stirred at room temperature for 1 hr. then the solvent evaporated to yield the desired product as white solid. The product was purified by HPLC (C-18) using a 20-50% acetonitrile (0.1% (v/v) TFA)/aqueous( 0.1% (v/v) TFA) gradient elution to give the two product diastereomers. In a HPLC elution system (C-18, 0-50% acetonitrile (0.1% (v/v) TFA)/aqueous( 0.1% (v/v) TFA) gradient elution, diastereomer fast is the fast moving compound (0.725

g) while diastereomer slow is the slower moving (0.687 g) (overall yield, 71%).

$^1$H NMR (methanol-$d_4$) δ: Diastereomer Fast(rt=20.57) 1.10 (m, 2H), 1.42 (m, 1H), 1.58 (m,1H), 1.80 (m, 2H), 2.28 (s 6H), 2.62 (t, 2H, J=7.8 Hz), 3.02 (m, 3H), 3.18 (m, 3H), 3.94 (dd, 1H, J=11.6 Hz and 4.8 Hz), 4.08 (dd, 1H, J=5.2 and 8.9 Hz), 6.53 (s 2H), 7.13-7.27 (m, 5H), 8.08 (t, 1H, J=5.5 Hz); MS : (m/e) 482.8. Diastereomer Slow(rt=22.08), 1.55 (m, 3H), 1.78 (m, 3H), 2.25 (s 6H), 2.64 (t, 2H, J=7.8 Hz), 3.02 (dd, 1H, J=5.3 and 13.9 Hz)4.31 (t, 1H, J=6.7 Hz), 6.48 (s 2H), 7.15-7.39 (m, 5H), 7.55 (t, 1H, J=5.6 Hz); MS: (m/e) 483.0

EXAMPLE 8 2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoic acid (2-hydroxy-3-phenyl-propyl)amide (compound 7)



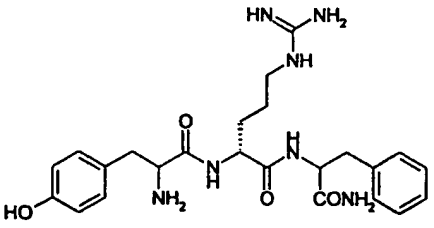

(7)

Compound (7) was prepared according to the same procedures used in example 7 with the exception that the intermediate (R)-1-amino-3-phenyl-propan-2-ol was used in place of phenylpropylamine in step 1. Intermediate (R)-1-amino-3-phenyl-propan-2-ol was prepared as follows.

(R)-2-benzyl-oxirane (5.02 g, 37.13 mmol) in aqueous amonium hydroxide (25%) (80 mL) was stirred for 72 hr at room temperature. Water was evaporated and the residue distilled under reduced pressure (5 mm Hg) at 140°C to yield the desired intermediate as white solid (3.24 g, 58%).

$^1$H NMR ($CDCl_3$) δ: 7.30 (m, 5 H), 3.73 (m, 1 H), 2.78 (m, 3 H), 2.56 (dd, 1 H).

EXAMPLE 9 H-TYR-[D]Arg-Phe-$NH_2$ (compound 8)



(8)

The synthetic peptide was prepared using Knorr resin functionalized with the relevant C-terminal N- Fmoc-amino acid residue (phenylalanine). All amino acids had their alpha amino group Fmoc protected and the following side chains: Pmc for [D]arginine and tBu for tyrosine.

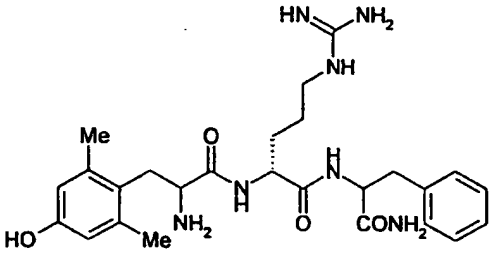
Dimethylformamide was of American Chemical Standards grade purity and used without further purification. TFA was of biograde purity. $H_20$ and acetonitrile were HPLC grade solvents. All remaining solvents were of A.C.S. grade purity and used as such without any purification. All Fmoc protected amino acids were obtained from Genzyme Pharmaceuticals or Novabiochem USA.

Solid phase peptide synthesis was carried out manually on Knorr resin. Resin loading was in the order of 0.84 mmoles/g and synthesis was performed on a 3.36 mM scale. Peptide condensation was carried out using 2 equivalents each of Fmoc-AA, HOBT, BOP or DCC and 4 equivalents of N-methylmorpholine (with BOP) in DMF for 2-18 hours at room temperature. The N- Fmoc deprotections steps were carried out using 20% (v/v) piperidine in DMF for 25 minutes.

The removal of side chain protecting groups (tBu, Pmc) and cleavage of the peptide from the resin was accomplished by using TFA containing scavenger:(cocktail- 55/2.5/2.5/40 TFA/anisole/EDT/DCM for 90 minutes at room temperature under nitrogen). The solvents were removed by evaporation and the peptide precipitated from diethyl ether, filtered, air dried and then dissolved in 10% (v/v) acetic acid/water and lyophilized.

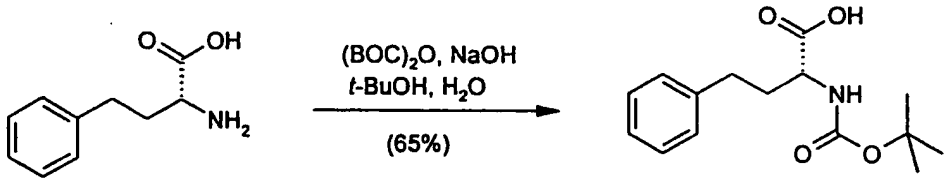
The crude peptide was purified and analyzed by HPLC on a reversed phase column (Vydac, 10 micron, 300A) using a flow rate of 9 ml/min with a gradient elution using water plus 0.06% TFA and acetonitrile plus 0.06% TFA in a 0-100% gradient over 80 minutes. The pure fractions were combined and lyophilized giving the pure peptide in the trifluoroacetic acid salt form.

In a like manner the following compound may also be prepared: dimethyltyrosine-[D]Arg-Phe-$NH_2$ (compound 9);

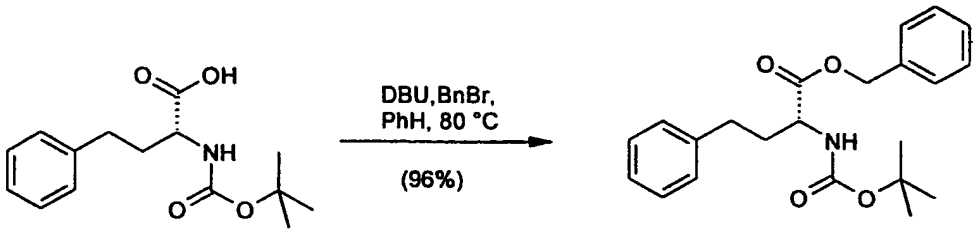



(9)

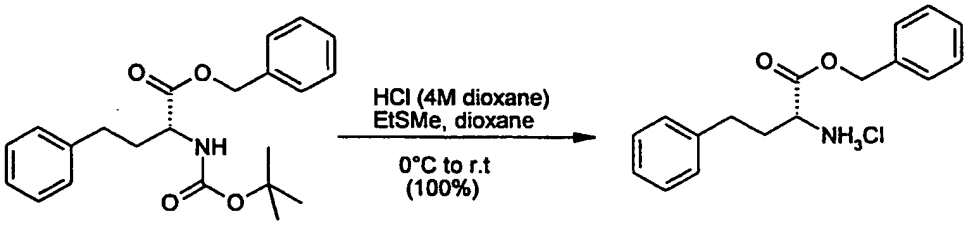
EXAMPLE 10 2-{2-[2-aminomethyl-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoylamino}-4-phenyl-butyric acid (compound 10)



To a solution of NaOH (0.4 g, 10.98 mmol) in $H_2O$ (11 mL), was added the D-homophenylalanine (1.788 g, 9.98 mmol) and *t*-BuOH (5 mL) at rt. A solution of $(BOC)_2O$ (2.286 g, 10.45 mmol) in t-BuOH (3 mL) was added over a period of 10 min. and then stirred over night. The reaction mixture was extracted with $CH_2Cl_2$ (2x30 mL), the combined organic layer washed with sat. $NaHCO_3$ (3x20 mL) and the aqueous layer acidified with HCl (1N) at 0 °C, then washed with AcOEt (3x200 mL). The organic layer was washed with $H_2O$, brine, dried over $MgSO_4$ and then evaporated to give desired product (1.82 g, 65 %).

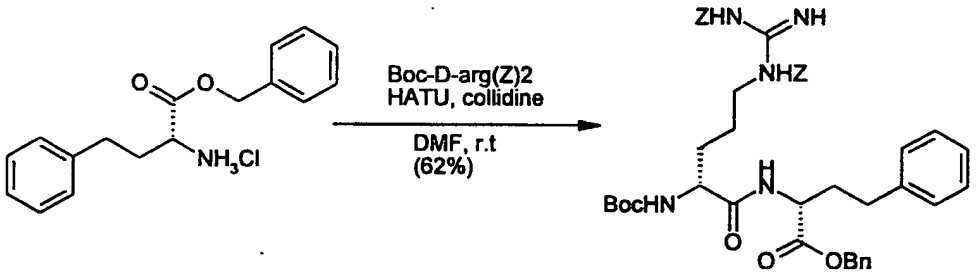



The acid (1.82 g, 6.52 mmol), DBU (974 µL, 6.52 mmol) and benzylbromide (1.70 mL,9.77mmol) in benzene (40 mL) were heated at reflux for 3h. The DBUxHBr was filtered and washed with AcOEt (400 mL). The organic layer was washed with sat. $NaHCO_3$ (1x50 mL), citric acid (0.5M)(1x50 mL), $H_2O$ (1x 50 mL), brine and dried over $MgSO_4$. The crude materill was purified by a flash chromatography (AcOEt/Hex, 1:4) to give the desired product (2.29 g, 96%).

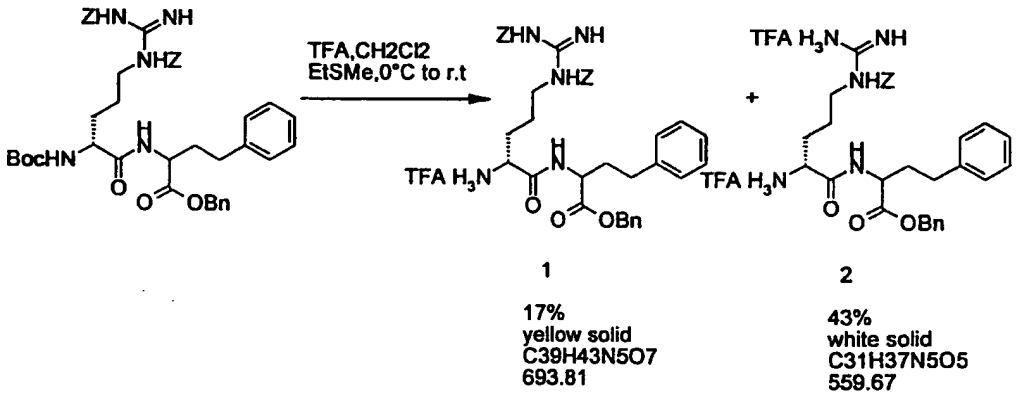



To a solution of the Boc derivative (2.237 g, 6.05 mmol) in dioxane (10 mL) at 0 °C was added the ethylmethylsulfide (1.5

mL) and HCl (4M in dioxane)(20 mL). The solution was stirred at 0 °C for 30 min and then allowed to warm to rt. The volatile was removed and the white solid was dried in vacuo for 3h. (1.85g, 100%).

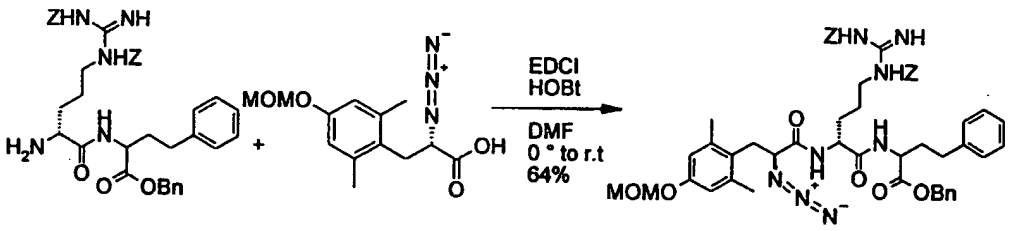



To a solution of the amine salt (1.85 g, 6.05 mmol), Boc-D-Arg(Z)$_2$ (2.983 g, 5.5 mmol)in DMF (10 mL) was added the 2,4,6-collidine (0.8 mL, 6.06 mmol) and 0-(7-aza-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) (2.76 g, 7.26 mmol) at 0 °C. After 30 min at 0 °C, the solution was stirred at rt for 16 h. The solution was diluted with AcOEt (400 mL) and washed in sequence with saturated $NaHCO_3$ (2x50 mL), $H_2O$(1x 50 mL), citric acid (2x 50 mL), $H_2O$ (1x 50 mL), brine (2x 50 mL) and dried over $MgSO_4$. The product was purified by flash chromatography (AcOEt/Hex, 2:3)(2.962 g, 62%).

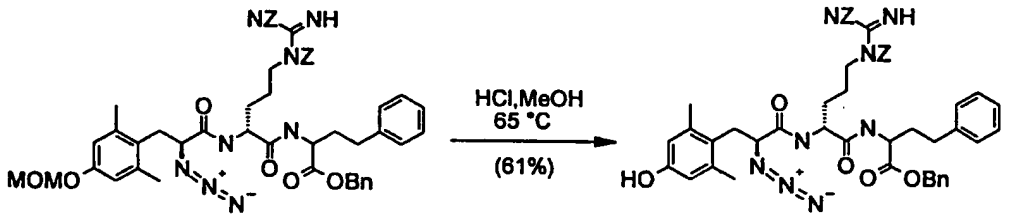



To a solution of Boc-D-arg-ω,ω'(Z)$_2$-Homophe-OBz (2.962 g, 3.74 mmol) in $CH_2Cl_2$ (30 mL) was added the ethylmethylsulfide (2 mL), and TFA (7 mL) at 0 °C. After 30 min at 0 °C, the solution was

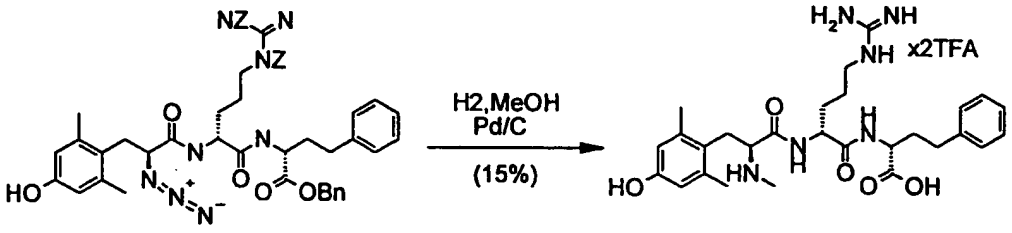
stirred at rt for 3h. The solution was diluted with AcOEt (400 mL) and washed with saturated $NaHCO_3$ (2x 50 mL), $H_2O$(1x 50 mL), brine (1x 50 mL) and dried over $MgSO_4$. The product was purified by flash chromatography ($MeOH/CH_2Cl_2/NEt_3$, 3:95:2)(446 mg of 1 and 895 mg of 2).




To a solution of Boc-D-arg-ω,ω'$(Z)_2$-Homophe-OBz TFA salt (226 mg, 0.326 mmol) and azidoacid (96 mg, 0.34 mmol) in DMF (4 mL) was added the 1-hydroxybenzotriazole (HOBt) (66 mg, 0.49 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) (94 mg, 0.49 mmol) at 0 °C. After 30 min at 0 °C, the solution was stirred at rt for 16 h. The solution was diluted with AcOEt (400 mL) and washed in sequence with saturated $NaHCO_3$ (2x 50 mL), $H_2O$ (1x 50 mL), citric acid (2x 50 mL), $H_2O$ (1x 50 mL), brine (2x 50 mL) and dried over $MgSO_4$. The product was purified by flash chromatography (AcOEt/Hex, 3:5 to 1:1)(198 mg, 64%)




To a solution of $Z_2$-derivative (198 mg, 0.207 mmol) in MeOH (10 mL) and one drop of concentrate HCl. After 30 min at reflux, the solution was cooled to rt, diluted with AcOEt (400 mL) and washed with saturated $NaHCO_3$ (2x), $H_2O$, brine and dried over $MgSO_4$. The product was purified by flash chromatography (AcOEt/Hex, 4:6 to 1:1)(115 mg, 61%).

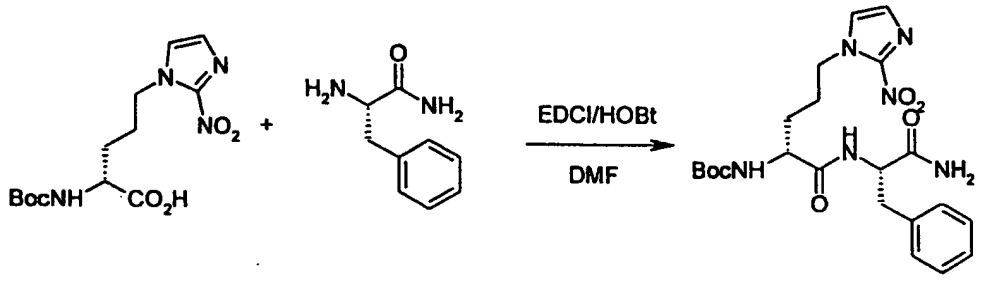



(10)

A solution of the azide (115 mg, 0.126 mmol) in MeOH (3 mL), Pd/C (19.0 mg) and $H_2$ (1atm) was stirred over night. The catalyst was filtered and the solvent evaporated. The compound was purified by HPLC. (10mg, 15%)

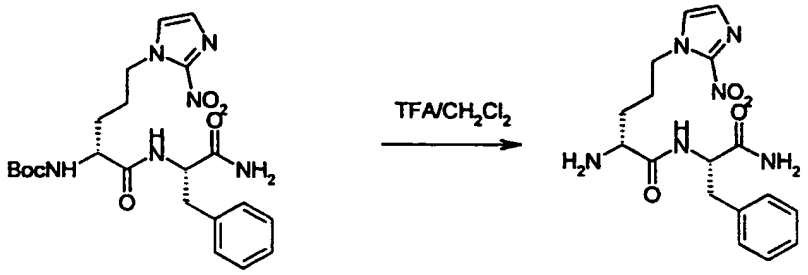
$^1$H NMR ($CD_3OD$): 8.63 (1H, d, J=7.5Hz, NH), 7.30-7.15 (5H, m, H-Ar), 6.54 (2H, s, H-Ar of DMT), 4.33 (1H, q,J=5Hz, NCH),4.22 (1H, q,J=5.5Hz, NCH), 3.93 (1H, dd, J=5Hz and 12Hz, NCH), 3.69 (3H, s, $CH_3N$), 3.26 (2H, t, J=12.0Hz), 3.10-3.00 (3H, m), 2.80-2.65 (2H, m), 2.29 (6H, s, $CH_3$), 2.17 (1H, m), 1.99 (1H, m), 1.64 (1H, m), 1.44 (1H, m), 1.35-1.20 (2H,m).

EXAMPLE 11 N1-[(1S)-1-Carbamoyl-2-phenylethyl]-(2R)-5-(2-amino)-1H-1,3-diazol-1-yl)-2-[(1S0-1-amino-2-(4-hydroxy-2,6-dimethylphenyl)ethylcarboxamide)pentanamide (compound 11)

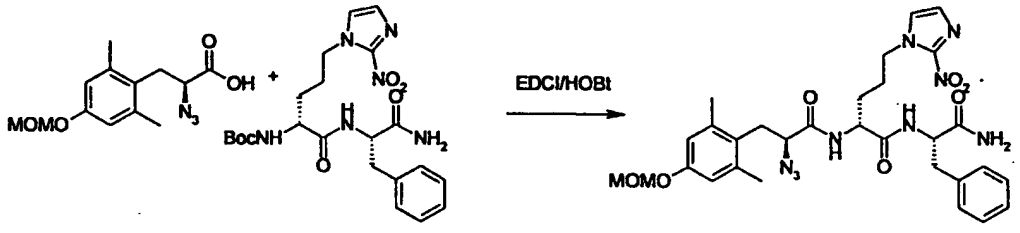



To a mixture of 2R-tert-Butoxycarbonylamino-5-(2-nitro-imidazol-1-yl)-pentanoic acid (0.268g, 0.816 mmol), HOBt (0.165 g, 1.224 mmol) and S-phenylalaninamide(0.134 g, 0.816 mmol) in DMF (10

ml) was added EDCI (0.235 g, 1.224 mmol) at 0°C. The solution was warmed to ambient temperature and stirred overnight. DMF was evaporated and the residue partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give the desired product as white solid (0.36 g, 92%).

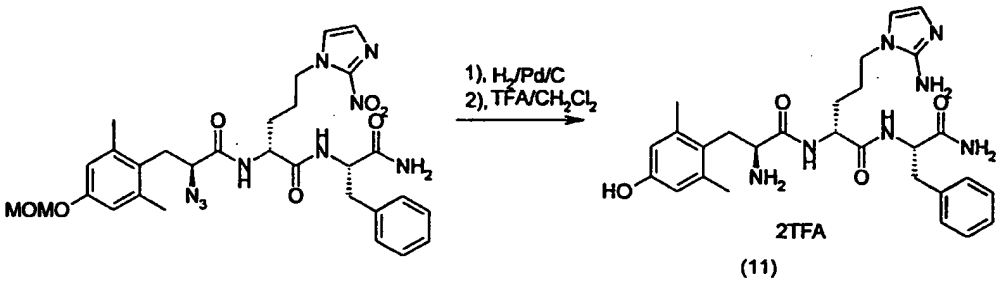



[4-(2-nitro-imidazol-1-yl)-1S-(carbamoyl-2-phenyl-ethylcarbamoyl)]-carbamic acid tert-butyl ester (0.360 g, 0.759 mmol) was dissolved in $TFA/CH_2Cl_2$ (1:1) (10 mL) and stirred at room temperature for 1 hr. The solvent was evaporated. The residue was dissolved in ethyl acetate, washed with sat. $NaHCO_3$ aqueous solution and brine, dried over $Na_2SO_4$, filtered, and the filtrate was evaporated to yield the desired product as oil (0.190 g, 68%).




To a mixture solution of 2R-Amino-5-(2-nitro-imidazol-1-yl)-pentanoic acid (1S-(carbamoyl-2-phenyl-ethylcarbamoyl)-amide (0.090 g, 0.24 mmol), HOBt (0.049 g, 0.36 mmol) and (S)2,6-Me2Tyr(N3)-OH (0.067 g, 0.24 mmol) in DMF (3 ml) was added EDCI

(0.465 g, 2.43 mmol) at 0°C. Then it was warmed to ambient temperature and stirred overnight. DMF was evaporated. The residue was partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtered. The filtrate was evaporated to give the desired product as white solid (0.15g, 75%).

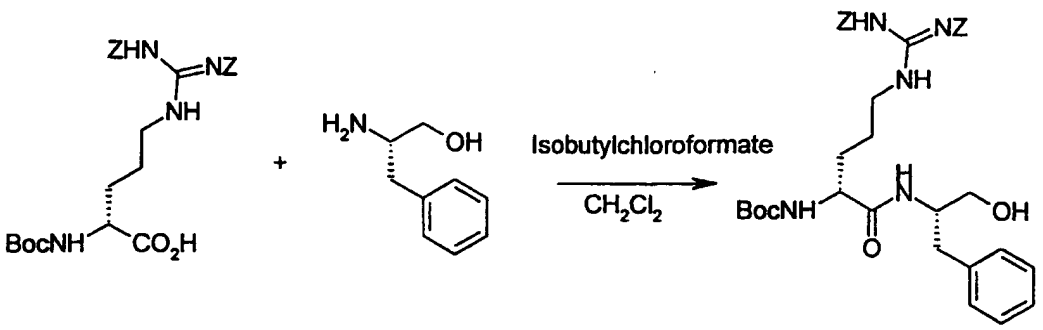



(11)

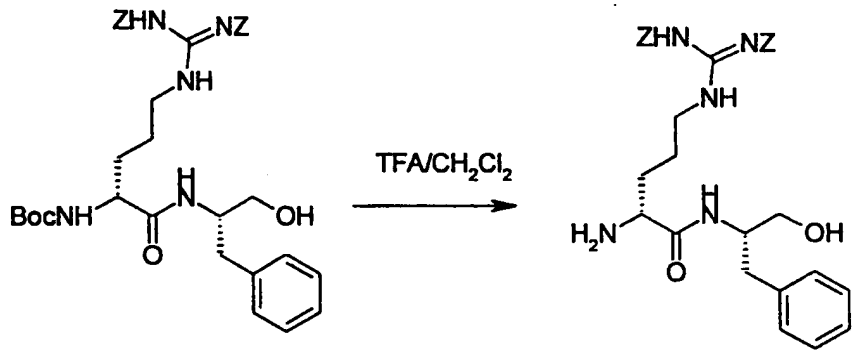
[1-[1R-(1S-carbamoyl-2-phenyl-ethylcarbamoyl)-4-(2-nitro-imidazol-1-yl-butylcarbamoyl]-2-(4-hydroxy-2,6-dimethyl-phenyl)-2-azidoethyl] (0.046 g, 0.072 mmol) was dissolved in methanol (5 ml). Pd/C (0.010 g) was added. The solution was stirred under hydrogen for 2 hr. Catalyst was filtered off. the filtrate was evaporated. The residue was dissolved in TFA/CH2Cl2 (1:1, 2 ml) and stirred for 30 min. solvent was evaporeted. The residue was purified by HPLC using a gradient A/B (0 to 70/30) ( A: 0.1% (v/v) TFA aqueous, B: 0.1% (v/v) acetonitrile), followed by lyphylination of aquous solution to give the desired product as white powder (0.048 g, 87%).

$^1H$ NMR ($CD_3OD$ ) δ:0.95 (m, 1H), 1.12 (m, 2H), 1.25 (m, 1H), 2.25 (s, 6H), 2.75 (m, 1H), 2.95 (m, 1H), 3.15 (m, 1H), 3.31 (m, 1H), 3.52 (m, 2H), 3.85 (m, 1H), 4.02(m, 1H), 4.62 (m, 1H), 6.47 (s, 2H), 6.64(s, 1H), 6.85 (s, 1H), 7.05-7.25 (m, 5H).

EXAMPLE 12 (2R)-[(2S)-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoic acid ((1S)-benzyl-2-hydroxy-ethyl)-amide, bistrifluoroacetic acid salts (compound 12)

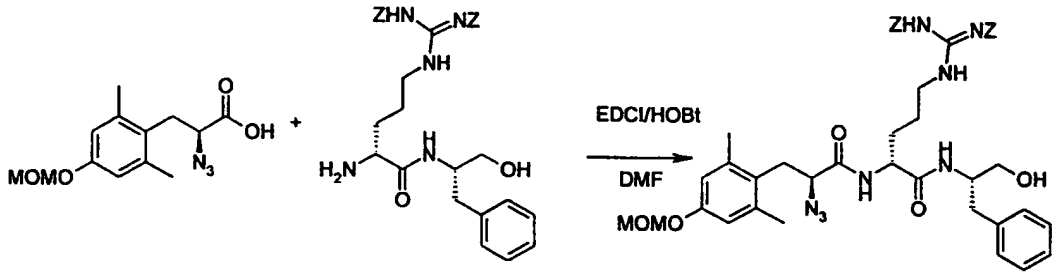



Isobutylchloroformate (0.0729 g, 0.535mmol) was added to a solution of Boc-D-Arg($Z_2$)-L-Phe-OBzl (0.300 g, 0.535 mmol) and triethylamine (0,065 g, 0.642 mmol) in dichloromethane (5 ml) at 0°C. The mixture was stirred for 1 hr. (s)-(-)20Amino-3-phenyl-1-propanol (0.081 g, 0.535 mmol) was added. The reaction mixture was then warmed to room temperature and stirred for 1 hr. Dichloromethane (20 ml ) was added, washen with 10% $KHSO_4$ aqueous solution, saturated $NaHCO_3$ aqueous solution, brine, dried, and filtered. The filtrate was evaporated to give the desired product as oil (0.35 g, 95%).

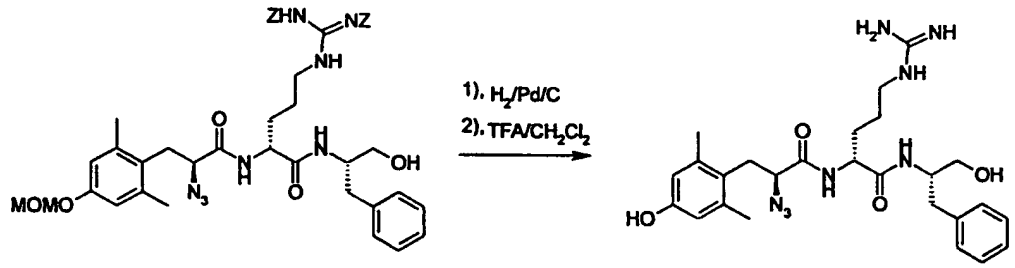



Boc-D-Arg($Z_2$)-L-(2-amino-3-phenyl-1-propanol) (0.350 g, 0.504 mmol) was dissolved in TFA/$CH_2Cl_2$ (1:1) (10 mL). The solution was stirred at room temperature for 1 hr. The solvent was evaporated. The residue was dissolved in ethyl acetate, washed with sat. $NaHCO_3$ aqueous solution and brine, dried over $Na_2SO_4$,

filtered, and thye filtrate was evaporated to yield the desired product as oil (0.320 g, 97%).




To a mixture solution of D-Arg($Z_2$)-L-(2-amino-3-phenyl-1-propanol)(0.320g, 0.475 mmol), HOBt (0.163g, 0.243 mmol) and MOMO-DMT(N3)-OH(0.225g, 0.805 mmol) in DMF (10 ml) was added EDCI (0.231 g, 1.208 mmol) at 0°C. Then it was warmed to ambient temperature and stirred overnight. DMF was evaporated. The residue was partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtered. The filtrate was evaporated to give the desired product as white solid (0.200 g, 51%).



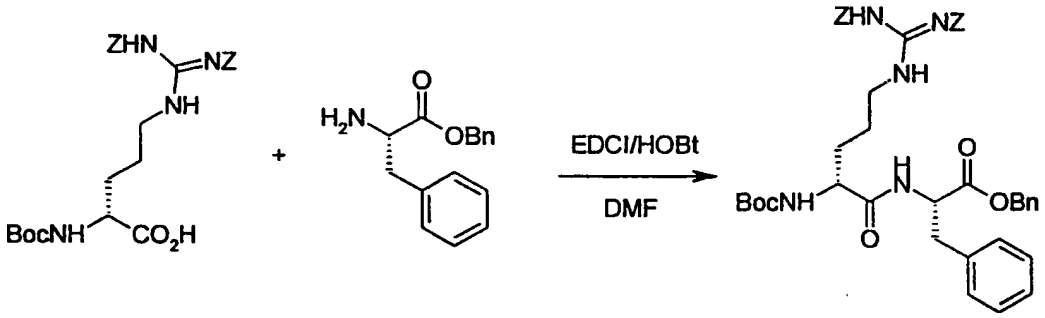

(12)

MOMO-DMT($N_3$)- D-Arg($Z_2$)-L-(2-amino-3-phenyl-1-propanol)(0.200 g, 0.244 mmol) was dissolved in methanol (10 ml). Pd/C (0.015 g) was added. The solution was stirred under hydrogen for 2 hr. Catalyst was filtered off. the filtrate was evaporated. The residue was dissolved in TFA/CH2Cl2 (1:1, 5 ml) and stirred for 30 min. Solvent was evaporeted. The residue was purified by HPLC using a gradient A/B (0 to 50%) ( A: 0.1% (v/v) TFA aqueous, B:

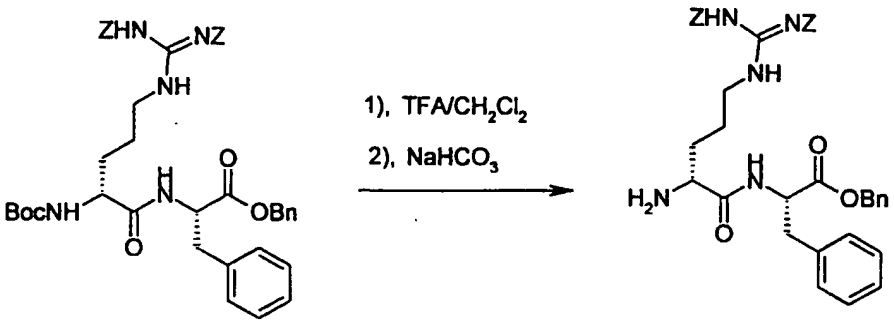
0.1% (v/v) acetonitrile), followed by lyphylination of aquous solution to give the desired product as white powder (0.150 g, 85%).

$^1$H NMR ($CD_3OD$) δ: 0.88 (m, 2H), 1.15(m, 1H), 1.30(m, 1H), 2.22(s, 6H), 2.63(m, 1H), 2.80-3.00 (m, 5H), 3.15(m, 1H), 3.55 (m, 2H), 3.85 (m, 1H), 4.02(m, 1H), 4.15(m, 1H), 6.61(s, 2H), 7.25 (m, 5H).

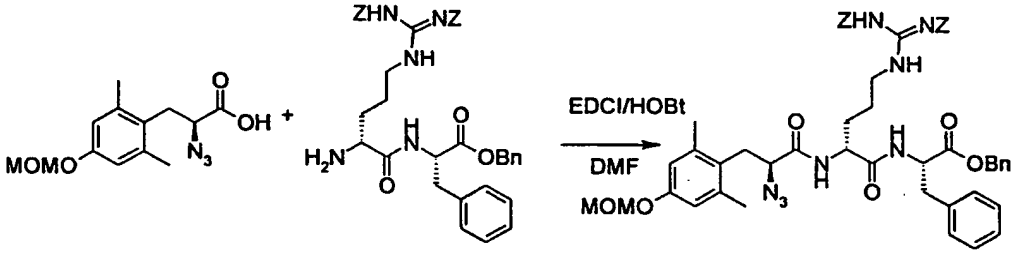
EXAMPLE 13 2S-{2R-[2S-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoylamino}-3-phenyl-propionic acid, bistrifluoroacetic salts (compound 13)



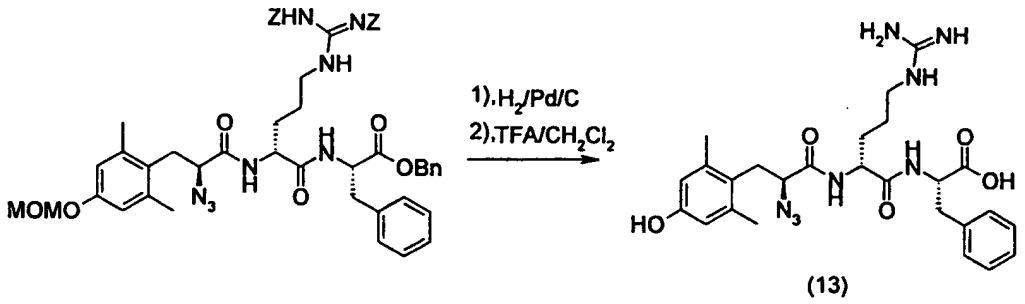

To a mixture solution of Boc-D-Arg(Z2)-OH (0.50 g, 0.892 mmol), HOBt (0.133 g, 0.981 mmol) and H-Phe-OBzl.HCl (0.26g, 0.892 mmol) and triethylamine (0.099 g, 0.981 mmol, 0.137 mmol) in DMF (10 ml) was added EDCI (0.188 g, 0.981 mmol) at 0°C. Then it was warmed to ambient temperature and stirred overnight. DMF was evaporated. The residue was partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtered. The filtrate was evaporated to give the desired product as white solid (0.70 g, 97%).




Boc-D-Arg(Z2)-L-Phe-OBzl (0.700 g, 0.878 mmol) was dissolved in $TFA/CH_2Cl_2$ (1:1) (10 mL). The solution was stirred at room temperature for 1 hr. The solvent was evaporated. The residue was dissolved in ethyl acetate, washed with sat. $NaHCO_3$ aqueous solution and brine, dried over $Na_2SO_4$, filtered, and thye filtrate was evaporated to yield the desired product as oil (0.57 g, 95%).




To a mixture solution of D-Arg(Z2)-L-Phe-OBzl(0.57 g, 0.839 mmol), HOBt (0.125g, 0.923 mmol) and MOMO-DMT(N3)-OH(0.234g, 0.839 mmol) in DMF (10 ml) was added EDCI (0.177 g, 0.923 mmol) at 0°C. Then it was warmed to ambient temperature and stirred overnight. DMF was evaporated. The residue was partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtered. The filtrate was evaporated to give the desired product as white solid (0.609 g, 77%).

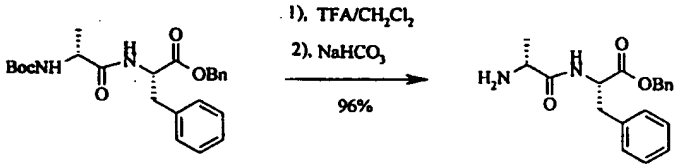



(13)

MOMO-DMT(N3)- D-Arg(Z2)-L-Phe-OBzl (0.60 g, 0.638 mmol) was dissolved in methanol (10 ml). Pd/C (0.068 g) was added. The solution was stirred under hydrogen for 2 hr. Catalyst was filtered off. the filtrate was evaporated. The residue was dissolved in TFA/CH2Cl2 (1:1, 5 ml) and stirred for 30 min. Solvent was evaporeted. The residue was purified by HPLC using a gradient A/B (10 to 50%) ( A: 0.1% (v/v) TFA aqueous, B: 0.1% (v/v) acetonitrile), followed by lyphylination of aquous solution to give the desired product as white powder (0.220 g, 45%).

EXAMPLE 14 2S-{3-[2R-Amino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino]-2R-methyl-propionylamino}-3-phenyl-propionic acid, trifluoroacetic acid salt(compound 14)

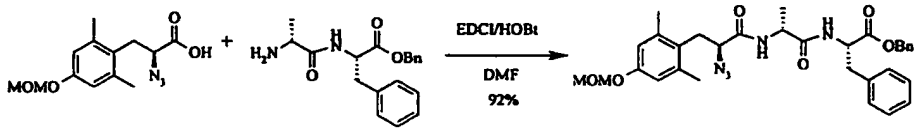
Step 1. Preparation of 2S-(3-Amino-2R-methyl-propionylamino)-3-phenyl-propionic acid benzyl ester




2S-(3-tert-Butoxycarbonylamino-2R-methyl-propionylamino)-3-phenyl-propionic acid benzyl ester (1.50g, 3.24 mmol) was dissolved in TFA/$CH_2Cl_2$ (1:1) (10 ml). The solution was stirred

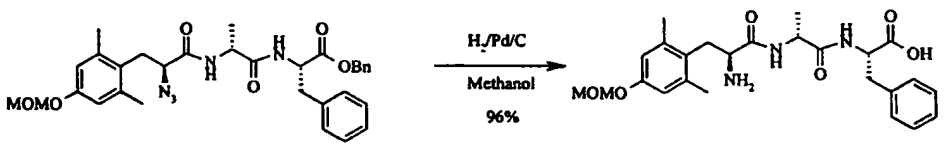
at room temperature for 1 hr. The solvent was evaporated. The residue was partitioned between ethylacetate and $NaHCO_3$ (Sat.) aqueous solution, separared, dried over $MgSO_4$, filtered. The filtrate was evaporated to give the desired product as oil. (1.00g, 96%).

Step 2. Preparation of 2S-{3-[2R-Azido-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino]-2R-methyl-propionylamino}-3-phenyl-propionic acid benzyl ester



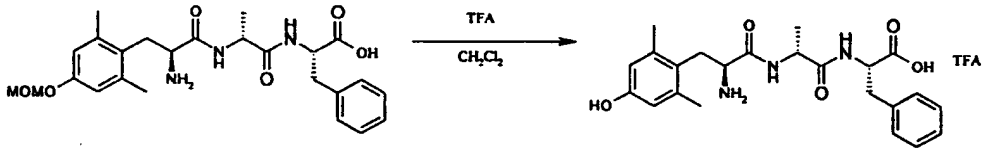

To a mixture solution of 2R-azido-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionic acid (0.25 g, 0.895 mmol), HOBt (0.145g, 1.074 mmol) and 2S-(3-Amino-2R-methyl-propionylamino)-3-phenyl-propionic acid benzyl ester (0.288g, 0.895 mmol) in DMF (10 ml) was added EDCI (0.206g, 1.074 mmol) at 0°C. Then it was warmed to ambient temperature and stirred overnight. DMF was evaporated. The residue was partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$ aqueous solution and brine, dried over $MgSO_4$, filtered. The filtrate was evaporated to give the desired product as white solid (0.48g, 92%).

Step 3 2S-{3-[2R-Amino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino]-2R-methyl-propionylamino}-3-phenyl-propionic acid



2S-{3-[2R-Azido-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino]-2R-methyl-propionylamino}-3-phenyl-propionic acid benzyl ester (0.350g, 0.600 mmol) was dissolved in methanol (10 ml) Pd/C (0.060 g, 10%) was added. The resulting mixture was stirred under hydrogen at ambient temperature for 1 hr. Catalyst was filtered off. The filtrate was evaporated to give the desired product as white solid (0.270 g, 96 %).

Step 4 Preparation of 2S-{3-[2R-Amino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino]-2R-methyl-propionylamino}-3-phenyl-propionic acid, trifluoroacetic acid salt

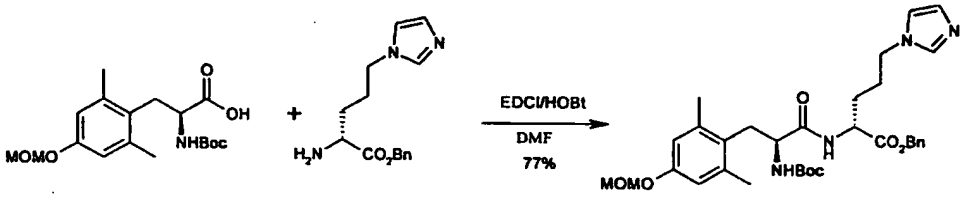



2S-{3-[2R-Amino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino]-2R-methyl-propionylamino}-3-phenyl-propionic acid (0.270g, 0.463 mmol) was dissolved in TFA/$CH_2Cl_2$ (1:1) (10 ml). The solution was stirred at room temperature for 1 hr. The solvent was evaporated to yield the desired product as white solid . The product was purified by HPLC (C-18) using a 20- 70% acetonitrile (0.1% (v/v) TFA)/aqueous( 0.1% (v/v) TFA) gradient elution, lyophylization of the aqueous solution to give the desired product as white powder (0.171 g, 55%).

$^1$H NMR (DMSO) δ: 9.02(br, 1H), 8.34(d, 1H, J=8.4Hz), 7.98(d, 1H, J=7.9Hz), 7.26-7.15(m, 5H), 6.37(s, 2H), 4.41(m, 1H), 4.38(m, 1H), 3.82(dd, 1H, J=4.6 and 10.8Hz), 3.06(dd, 1H, J=13.8 and 4.6Hz), 2.95(t, 1H, J=13.2 Hz), 3.82(m, 2H), 2.15(s, 6H), 0.60(d, 3H, J=6.9Hz).

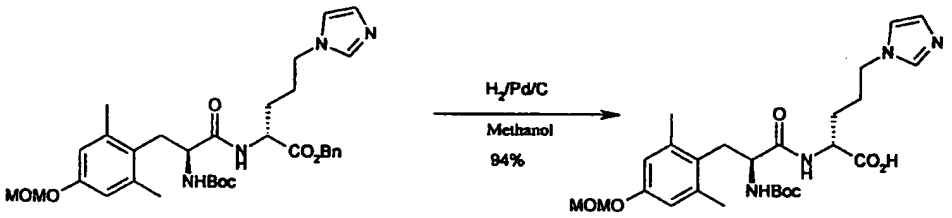
EXAMPLE 15 2S-{2R-[2S-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoylamino}-3-phenyl-propionic acid, trifluroroacetic acid salt (compound 15)

Step 1 Preparation of 2R-[2S-tert-Butoxycarbonylamino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoic acid benzyl ester



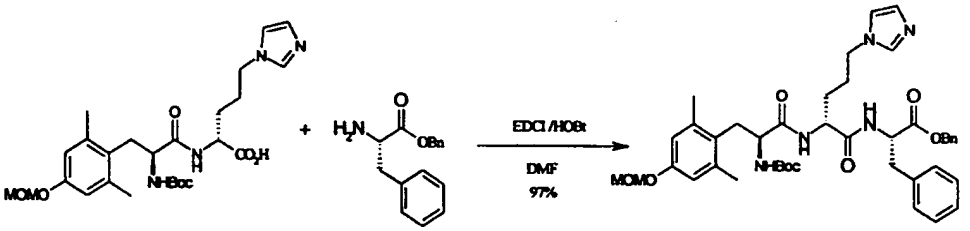

To a mixture solution of 2R-amino-5-imidazol-1-yl-pentanoic acid benzyl ester (0.761 g, 2.78 mmol), HOBt (0.563 g, 4.17 mmol) and 2S-tert-Butoxycarbonylamino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionic acid (0.750 g, 2.10 mmol) in DMF (10 ml) was added EDCI (0.799 g, 4.17 mmol) at 0°C. Then it was warmed to ambient temperature and stirred overnight. DMF was evaporated. The residue was partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$ aqueous solution and brine, dried over $MgSO_4$, filtered. The filtrate was evaporated to give the desired product as white solid (0.988 g, 77%).

Step 2 Preparation of 2R-[2S-tert-Butoxycarbonylamino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoic acid




2R-[2S-tert-Butoxycarbonylamino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoic acid benzyl ester (0.988 g, 1.61 mmol) was dissolved in methanol (10 ml) Pd/C (0.1720 g, 10%) was added. The resulting mixture was stirred under hydrogen at ambient temperature for 1 hr. Catalyst was filtered off. The filtrate was evaporated to give the desired product as white solid (0.790 g, 94%).

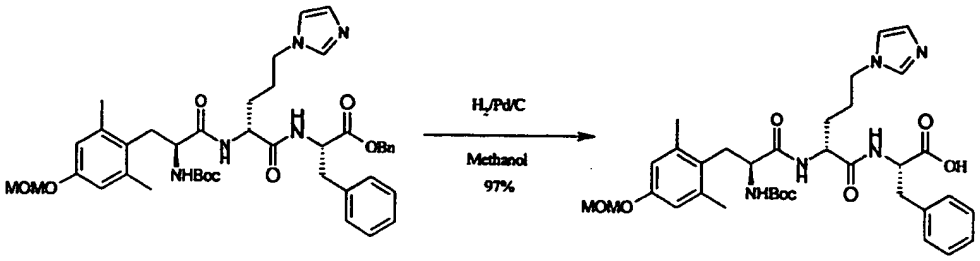
Step 3 Preparation of 2S-{2R-[2S-tert-Butoxycarbonylamino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoylamino}-3-phenyl-propionic acid benzyl ester




To a mixture solution of 2R-[2S-tert-Butoxycarbonylamino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoic acid (0.289 g, 0.553 mmol), HOBt (0.0897 g, 0.664 mmol), triethylamine (0.067 g, 0.664mmol), and S-Phe-OBn.HCl (0.161 g, 0.553mmol ) in DMF (10 ml) was added EDCI (0.127 g, 0.664 mmol) at 0°C. Then it was warmed to ambient temperature and stirred overnight. DMF was evaporated. The residue was partitioned between ethylacetate and water. The organic phase was washed with saturated $NaHCO_3$

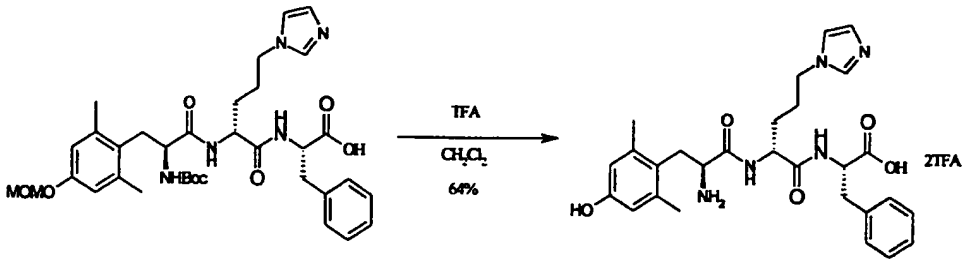
aqueous solution and brine, dried over $MgSO_4$, filtered. The filtrate was evaporated to give the desired product as white solid (0.406 g, 97%).

Step 4. Preparation of 2S-{2R-[2S-tert-Butoxycarbonylamino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoylamino}-3-phenyl-propionic acid




2S-{2R-[2S-tert-Butoxycarbonylamino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoylamino}-3-phenyl-propionic acid benzyl ester (0.406 g, 0.534 mmol) was dissolved in methanol (10 ml) Pd/C (0.0569 g, 10%) was added. The resulting mixture was stirred under hydrogen at ambient temperature for 1 hr. Catalyst was filtered off. The filtrate was evaporated to give the desired product as white solid (0.350 g, 97%).

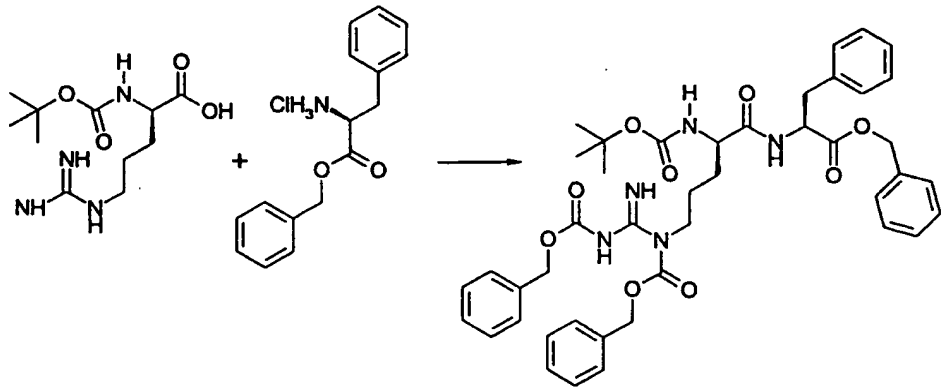
Step 5 Preparation of 2S-{2R-[2S-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoylamino}-3-phenyl-propionic acid, trifluroroacetic acid salt




2S-{2R-[2S-tert-Butoxycarbonylamino-3-(4-methoxymethoxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoylamino}-3-phenyl-propionic acid (0.350 g, 0.520 mmol) was dissolved in TFA/$CH_2Cl_2$ (1:1) (10 ml). The solution was stirred at room temperature for 1 hr. The solvent was evaporated to yield the desired product as white solid . The product was purified by HPLC (C-18) using a 20- 50% acetonitrile (0.1% (v/v) TFA)/aqueous( 0.1% (v/v) TFA) gradient elution, lyophylization of the aqueous solution to give the desired product as white powder (0.250 g, 64%).

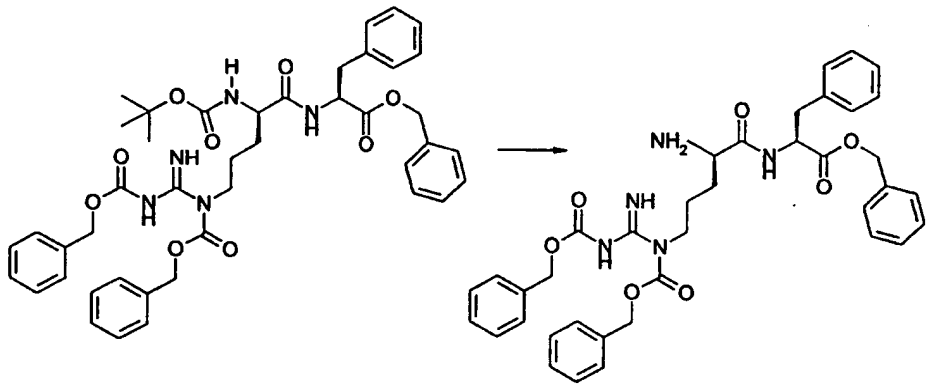
$^1$H NMR (DMSO) δ: 9.08(br, 1H), 8.80(br, 1H), 8.46(d, 1H, J=8.2Hz), 8.33(br, s, 3H), 8.27(d, 1H, J=8.2Hz), 7.15(m, 5H), 6.37(s, 2H), 4.45(m, 1H), 4.25(m, 1H), 3.85(m, 3H), 3.05(m, 2H), 2.75(m, 2H), 2.15(s, 6H), 0.95(m, 4H).

EXAMPLE 16 Me-Tyr-D-Arg-Phe-OH (compound 16)



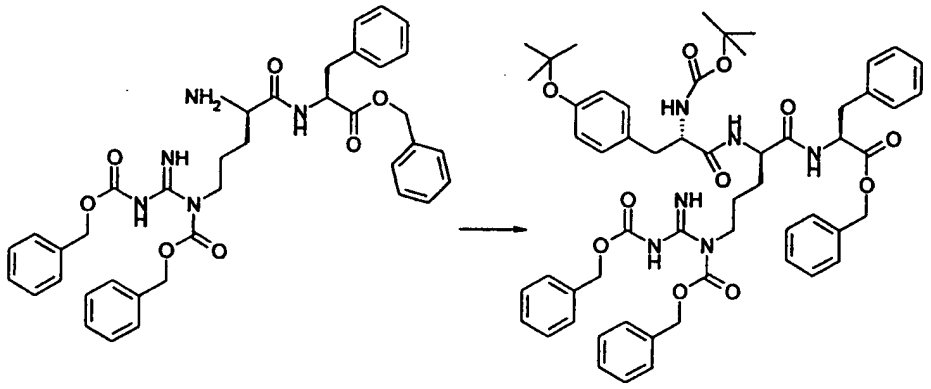
Step 1 Boc-D-Arg(Z)$_2$-Phe-OBz

A mixture of Phe-Obz.HCl (500 mg, 1.8 mmol.), N-Boc-D-Arg(Z)$_2$-OH (979.2 mg, 1.80 mmol.), 1-hydroxylbenzotriazole (243 mg, 1.89 mmol) 1-(3-dimethylpropyl)-3-ethylcarbodiimide hydrochloride (362.3 mg, 1.89 mmol) in 50 mL of dimethyl formamide was cooled to 0 °C. Triethylamine was added (250 µL, 1.80 mmol). The resulting mixture was stirred for 18 h as it warmed to r.t. The product was extracted from 15% NaCl solution using ethyl acetate. After removal of the solvent, a crude product was obtained. Chromatographic separation was complicated due to poor solubility of the product in the eluent (Hex :EtOAc=1 :1). Finally, 754 mg of product was obtained with good purity.



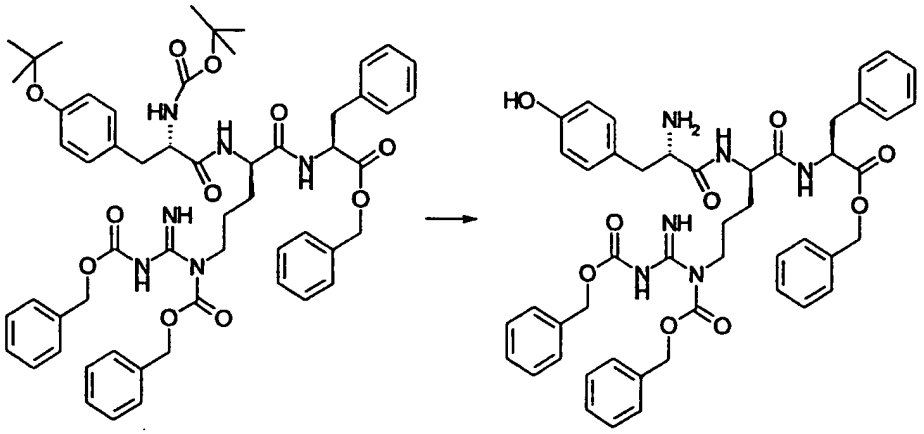
Step 2 H-D-Arg(Z)$_2$-Phe-O-Bz

A solution of the Boc derivative (174 mg, 0.223 mmol) in 3 mL of dioxane containing ethylmethyl sulfide (400 µL) was treated with HCl (4M in dioxane, 4 mL). After being stirred for 40 min at r.t., solvent was evaporated to give desired product.



Step 3 Boc-(O-t-Bu)Tyr-D-Arg(Z)$_2$-Phe-OBz

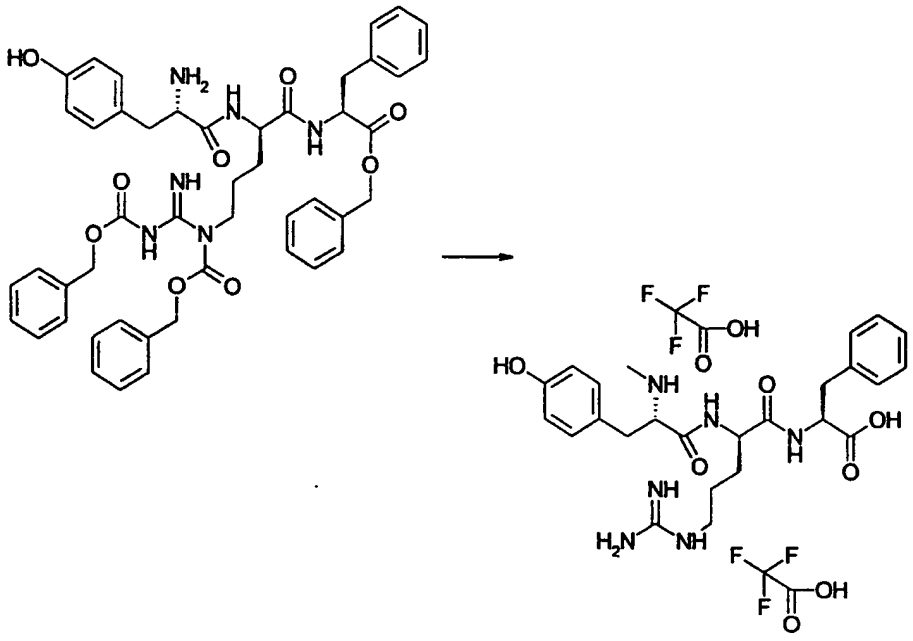
A mixture of (Z)$_2$dipeptide (277 mg, 0.41 mmol), Boc-(O-t-Bu)Tyr-OH (137 mg, 0.41 mmol), 1-hydroxylbenzotriazole (55.4 mg, 0.41 mmol), 1-(3-dimethylpropyl)-3-ethylcarbodiimide hydrochloride (78.6 mg, 0.41 mmol) in dichloromethane (12 mL) was cooled to 0 °C. Triethylamine (57.04 µL, 0.41 mmol) was added and the mixture was stirred for 18 h as it warmed to r.t. The crude product was chromatographed to give desired product (263 mg, 65.7%).



Step 4 H-Tyr-D-Arg(Z)$_2$-Phe-OBz

A solution of the tripeptide (373 mg, 0.380 mmol) stirred in 4 mL of dioxane containing ethylmethyl sulfide (0.5 mL) was treated with HCl (4.0 N in dioxane, 6 mL). The mixture was

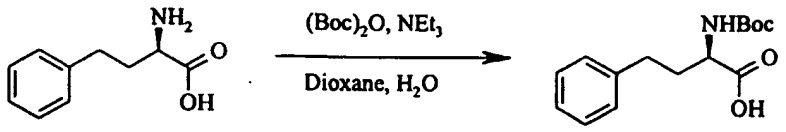
stirred for 1.5 h at r.t. then evaporated. Benzene (2X15 mL) was added and evaporated. The product was further subject to vacuum to remove the remaining solvent.



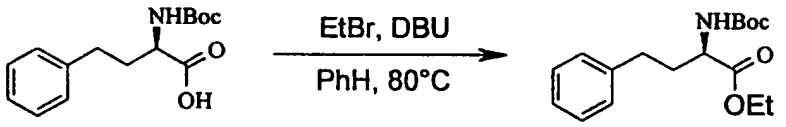
Step 5 Me-Tyr-D-Arg-Phe-OH

A methanolic solution of Z-protected tripeptide, with conc. HCl added (95 µL, 1.14 mmol), was catalytically hydrogenated under 1 atm of hydrogen (Pd/C, 120 mg) for 1.6 h at r.t. Solvent was evaporated to give a product from which was isolated using HPLC (0-50% aqueous acetonitrile/50 min) to give H-(N-Me)-Tyr-D-Arg-Phe-OH. 2 THA (61 mg). A mixture containing both products was also obtained (58 mg). The yield for this conversion was 96%. $^1$H NMR, DMSO-d6, δ, 1.04 (2H, m), 1.23 (1H, m), 1.30 (1H, m), 2.76-2.90 (4H, m), 3.07 (1H, dd, J1=5 Hz, J2=13 Hz), 4.02 (1H, m), 4.38 (1H, dd, J1=5 Hz, J2=7 Hz), 4.52 (1H, m), 6.70 (2H, d, J=9 Hz), 7.04 (2H, d, J=9 Hz), 7.20-7.28 (5H, m), 8.03 (2H, bs), 8.56 (1H, d, J=7.0 Hz), 8.69 (1H, d, J=7 Hz), 9.33 (1H, s).

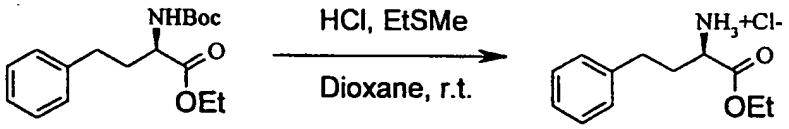
EXAMPLE 17 (S)-DMT-(OH)-D-Arg--D-Homophe-$OCH_3$ (compound 17)




Step 1 To a solution of D-Homophenylalanine (1.005g, 5.67 mmol) in dioxane (8mL) and $H_2O$ (10 mL) was added the triethylamine (1.6 mL, 11.34 mmol) and the $(Boc)_2O$ (1.49g, 6.81 mmol). The mixture was stirred at room temperature for over night. The solution was diluted with AcOEt (20 mL), the aqueous layer was acidified with HCl 10%, then washed with AcOEt (3x200 mL). The organic layer was washed with $H_2O$ (2x50 mL), brine (2x50 mL), dried over $MgSO_4$ and evaporated. The crude compound was used without any futher purification (1.467g, 93%).

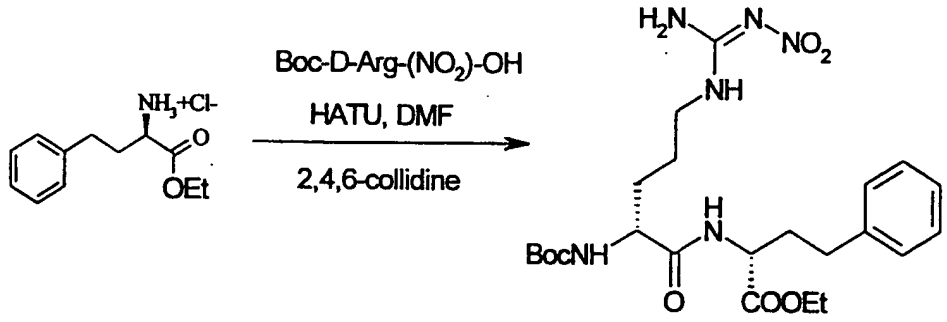



Step 2 The Boc-D-HomoPhe-OH (1.46 g, 5.25 mmol), DBU (0.785 mL, 10.5 mmol) and ethylbromide (0.785 mL,10.5 mmol) in benzene (10 mL) were heated at reflux for 3h.The DBUxHBr was filterated and washed with AcOEt (200 mL). The organic layer was washed with sat. $NaHCO_3$ (1x50 mL), citric acid (0.5M)(1x50 mL), $H_2O$ (1x 50 mL), brine and dried over $MgSO_4$. The desired product was obtained after evaporation of the solvent (1.44 g, 89%).

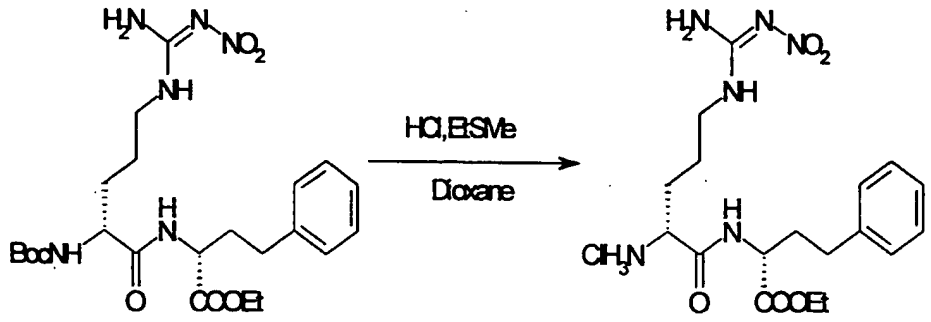



Step 3 To a solution of Boc -D-HomoPhe-OEt (1.43g, 4.67 mmol) in dioxane (22mL) at 0 °C was added the

ethylmethylsulfide (1.5 mL) and HCl (4M in dioxane)(10 mL). The solution was stirred at 0 °C for 30 min then was allowed to rt.. The volatile was removed and the yellow solid was dried in vacuo for 3h. (1.16 g, 100%).

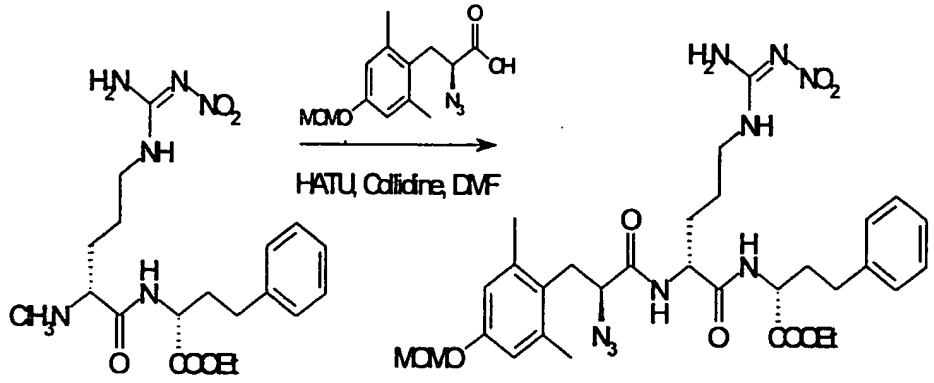



Step 4 To a solution of H-D-HomoPhe-OEt HCl salt (425 mg, 1.74 mmol), Boc-D-Arg($NO_2$)-OH (505 mg, mmol) in DMF (10 mL) was added the 2,4,6-collidine (1.2 mL, 9.0 mmol) and HATU (1.368 g, 3.6 mmol) at 0 °C. After 30 min at 0 °C, the solution was stirred at rt. for 16 h.The solution was diluted with AcOEt (400 mL) and washed in sequence with saturated $NaHCO_3$ (2x 50 mL), $H_2O$ ( 1x 50 mL),citric acid (2x 50 mL), $H_2O$ (1x 50 mL), brine (2x 50 mL) and dried over $MgSO_4$. The product was purified by a flash chromatography (AcOEt, 100%)(0.822 g, 97%)

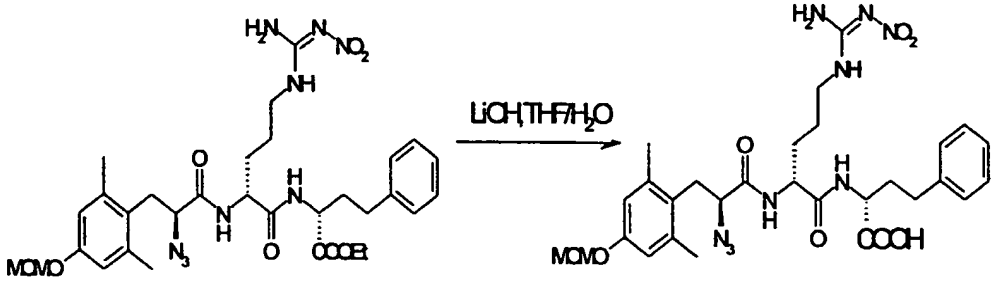



Step 5 To a solution of Boc -D-arg-D-HomoPhe-OEt (0.822 g, 1.62 mmol) in dioxane (4 mL) at 0 °C was added the ethylmethylsulfide (1.0 mL) and HCl (4M in dioxane)(4

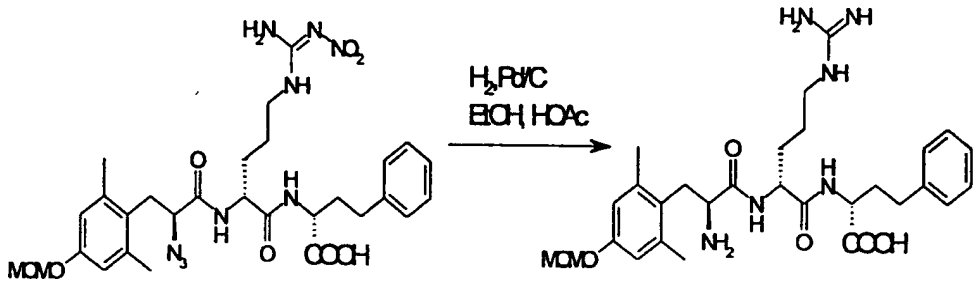
mL). The solution was stirred at 0 °C for 30 min then was allowed to rt.. The volatile was removed and the yellow solid was dried in vacuo for 3h. (0.721 g, 100%).



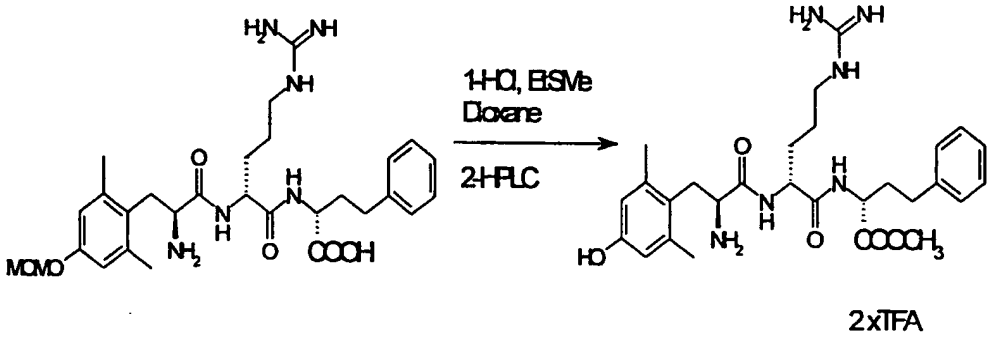

Step 6 To a solution of H-D Arg-($NO_2$)-D-HomoPhe-OEt HCl salt (721 mg, 1.62 mmol), ($N_3$)-DMT-(MOM)-OH (452 mg, 1.62 mmol) in DMF (5 mL) was added the 2,4,6-collidine (1.3 mL, 9.72 mmol) and HATU (1.23 g, 3.24 mmol) at 0 °C. After 30 min at 0 °C, the solution was stirred at rt. for 16 h.The solution was diluted with AcOEt (200 mL) and washed in sequence with saturated $NaHCO_3$ (2x 30 mL), $H_2O$ ( 1x 30 mL),citric acid (2x 30 mL), $H_2O$ (1x 30 mL), brine (2x 30 mL) and dried over $MgSO_4$. The product was purified by a flash chromatography (AcOEt, 100%)(0.927 g, 85%)



Step 7 To a solution of $(N_3)$-DMT-(MOM)-D Arg-$(NO_2)$-D-HomoPhe-Oet (322 mg, 0.5 mmol) in THF (5 mL) at 0 °C was added a solution of LiOH ( 83 mg, 1.98 mmol) in water (5 mL). The resulting mixture was stirred for 1h at 0°C. The solution was acidified with HCl 10%, then washed with AcOEt (2 x 60 mL). The organic layer was washed with brine, dried over $MgSO_4$.




Step 8 To a solution of $(N_3)$-DMT-(MOM)-D Arg-$(NO_2)$-D-HomoPhe-OH ( 320 mg, 0.5 mmol) in EtOH/ HOAc (4 :1 mL) was added the Pd/C (40 mg). The compound was hydrogenated at 45 psi for 36 h. The catalyst was filtered on celite, washed with EtOH and evaporated with toluene.



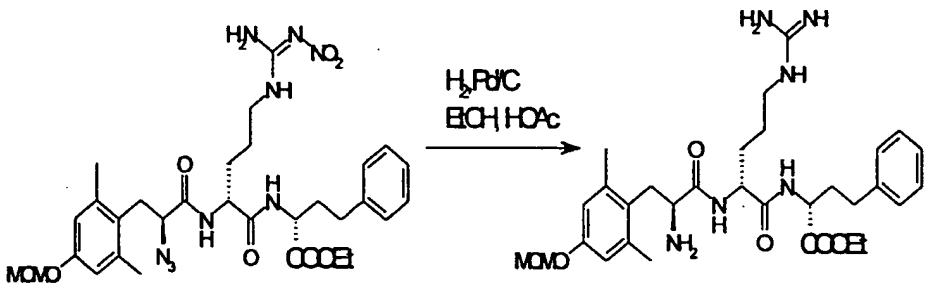

Step 9 To a solution of -DMT-(MOM)-D Arg--D-HomoPhe-OH ( 0.34 mmol) in dioxane (5 mL) was added the EtSMe (1.0 mL) and the HCl (4M in dioxane) (1.0 mL). The solution was stirred at romm temperature for 2h, then the solvant was

removed under vaccum. The crude material was purified by HPLC reversed phase (80 mg)

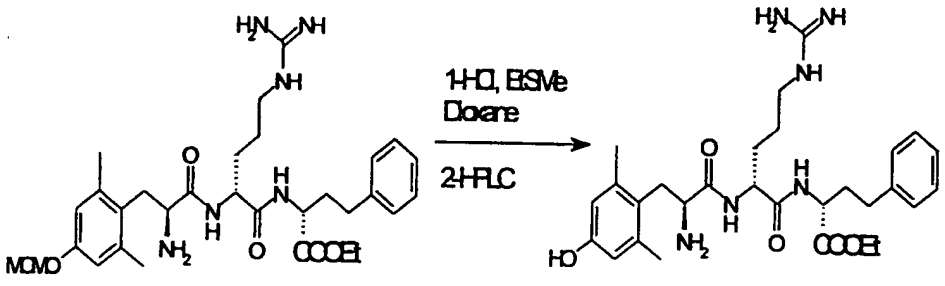
$^1$H NMR ($CD_3OD$): 8.62 (1H, d, J=7.5Hz, NH), 7.30-7.20 (5H, m, H-ar) , 6.54 (2H, s, H-ar of DMT), 4.33 (1H, m, $C\underline{H}COOCH_3$), 4.22 (1H, dd, J=5.5 Hz and 8.0 Hz, $NHC\underline{H}CO$), 3.93 (1H, dd,J=5.0Hz and 11.5 Hz, $C\underline{H}NH_2$), 3.68 (3H, s, $COOCH_3$), 3.25 (1H, m, $PhCH\underline{H}CHNH_2$), 3.10-3.00 (3H, m), 2.78 (1H, m, $PhCH\underline{H}$), 2.68 (1H, m, $PhC\underline{H}H$), 2.29 (6H, s, $CH_3$), 2.15 (1H, m), 1.65 (1H, m), 1.47 (1H, m), 1.35-1.20 (2H, m).

MS : 540.7 ($M^+$), 562.7 ($M^+$+Na)

EXAMPLE 18 (S)-DMT--D-Arg--D-Homophe-OEt (compound 18)




Step 1 To a solution of ($N_3$)-DMT-(MOM)-D Arg-($NO_2$)-D-HomoPhe-Oet, obtained as shown in steps 1 to 6 of example 17, ( 237 mg, 0.35 mmol) in EtOH/ HOAc (4 :1 mL) was added the Pd/C (30 mg). The compound was hydrogenated at 45 psi for 24 h. The catalyst was filtered on celite, washed with EtOH and evaporated with toluene.

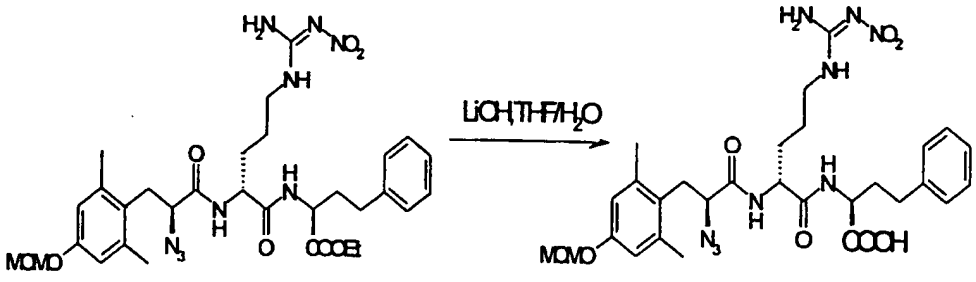



Step 2 To a solution of -DMT-(MOM)-D Arg--D-HomoPhe-OEt ( 209 mg, 0.35 mmol) in dioxane (5 mL) was added the EtSMe (1.0 mL) and the HCl (4M in dioxane) (1.0 mL). The solution was stirred at room temperature for 2h, then the solvant was removed under vaccum. The crude material was purified by HPLC reversed phase.

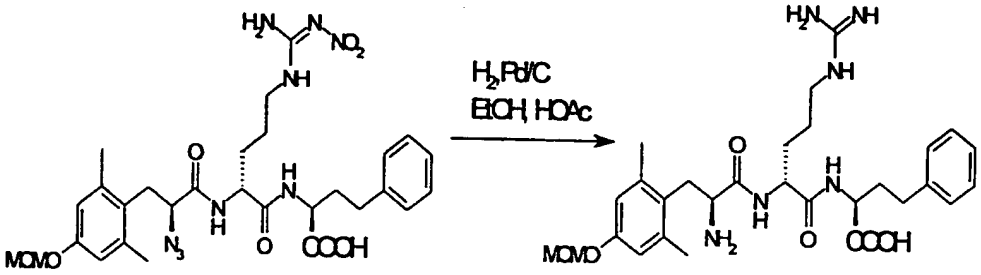
$^1$H NMR ($CD_3OD$): 7.30-7.20 (5H, m, H-ar) , 6.53 (2H, s, H-ar of DMT), 4.31 (1H, q, J=5.0Hz, CHNH), 4.21 (1H, t, J=5.5Hz, CHNH), 4.16 (2H, q, J=7.0Hz, $COOCH_2CH_3$), 3.92 (1H, dd, J=5.0 and 11.5Hz, $CHNH_2$), 3.24 (1H, t, J=11.5 Hz, $PhCHHCHNH_2$), 3.10-3.00 (3H, m), 2.77 (1H, m, PhCHH),2.70 (1H, m, PhHH), 2.29 (6H, s, $CH_3$), 2.13 (1H, m), 1.99 (1H, m), 1.65 (1H, m), 1.47 (1H, m), 1.35-1.30 (2H, m), 1.25 (3H, t, J=7.0Hz, $COOCH_2CH_3$).

MS : 554.7 ($M^+$), 576.6 ($M^+$+Na)

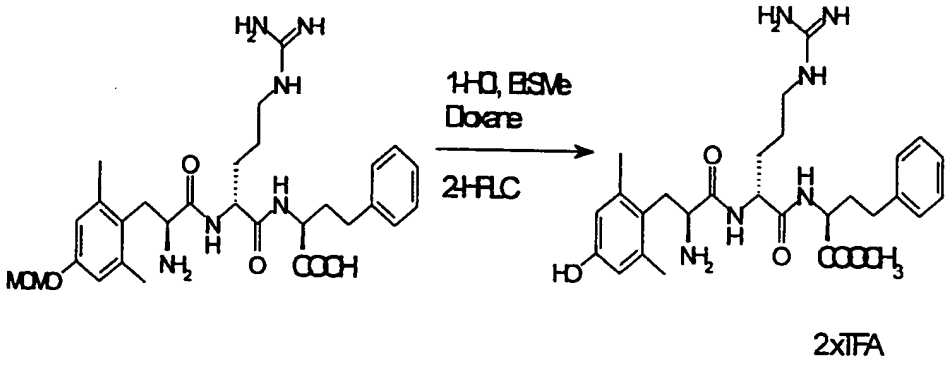
EXAMPLE 19 (S)-DMT-(OH)-D-Arg--L-Homophe-$OCH_3$ (compound 19)



Step 1 To a solution of $(N_3)$-DMT-(MOM)-D Arg-$(NO_2)$-L-HomoPhe-Oet (367 mg, 0.54 mmol), obtained in a similar manner as in Example 17, in THF (5 mL) at 0 °C was added a solution of LiOH ( 92 mg, 2.19 mmol) in water (5 mL). The resulting mixture was stirred for 1h at 0°C. The solution was acidified with HCl 10%, then washed with AcOEt (2 x 60 mL). The organic layer was washed with brine, dried over $MgSO_4$.




Step 2 To a solution of $(N_3)$-DMT-(MOM)-D Arg-$(NO_2)$-D-HomoPhe-OH ( 320 mg, 0.5 mmol) in EtOH/ HOAc (4 :1 mL) was added the Pd/C (40 mg). The compound was hydrogenated at 45 psi for 36 h. The catalyst was filtered on celite, washed with EtOH and evaporated with toluene.



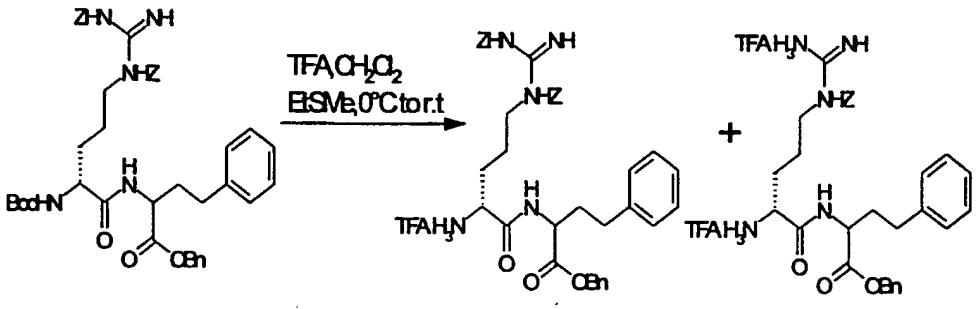

Step 3 To a solution of -DMT-(MOM)-D Arg-L-HomoPhe-OH (0.285 g, 0.5 mmol) in dioxane (5 mL) was added the EtSMe (1.0 mL) and the HCl (4M in dioxane) (1.0 mL). The solution was stirred at room temperature for 2h, then the solvant

was removed under vaccum. The crude material was purified by HPLC reversed phase (117 mg)

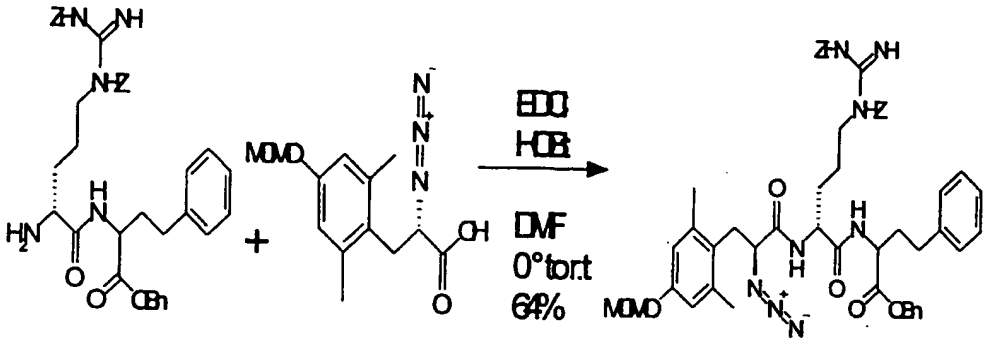
$^1$H NMR ($CD_3OD$): 7.30-7.20 (5H, m, H-ar) , 6.53 (2H, s, H-ar of DMT), 4.30 (2H, m ), 3.97 (1H, m ), 3.69 (3H, s, $COOCH_3$), 3.10-3.00 (3H, m), 2.75-2.55 (2H, m ), 2.30 (7H, m), 2.15 (1H, m), 2.05 (1H, m), 1.60 (1H, m), 1.47 (1H, m), 1.20 (2H, m).

MS : 540.7 ($M^+$), 562.7 ($M^+$+Na)

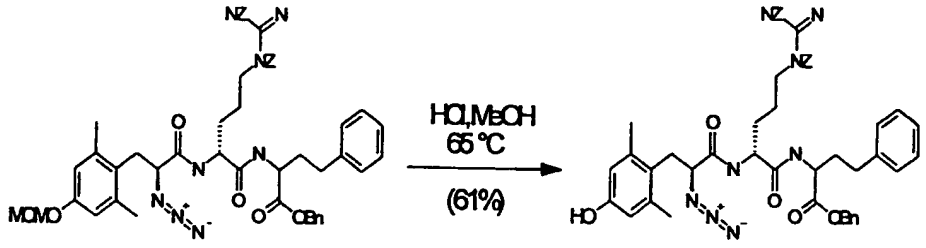
EXAMPLE 20 2-{2-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoylamino}-4-phenyl-butyric acid.
(compound 20)




**Procedure:** To a solution of Boc-D-arg-ω,ω'(Z)$_2$-Homophe-OBz (2.962 g, 3.74 mmol) in $CH_2Cl_2$ (30 mL) was added the ethylmethylsufide (2 mL), and TFA (7 mL) at 0 °C. After 30 min at 0 °C, the solution was stirred at rt. for 3h.The solution was diluted with AcOEt (400 mL) and washed with saturated $NaHCO_3$ (2x 50 mL), $H_2O$(1x 50 mL), brine (1x 50 mL) and dried over $MgSO_4$. The product was purified by a flash chromatography ($MeOH/CH_2Cl_2/NEt_3$, 3:95:2)(446 mg of 1 and 895 mg of 2).

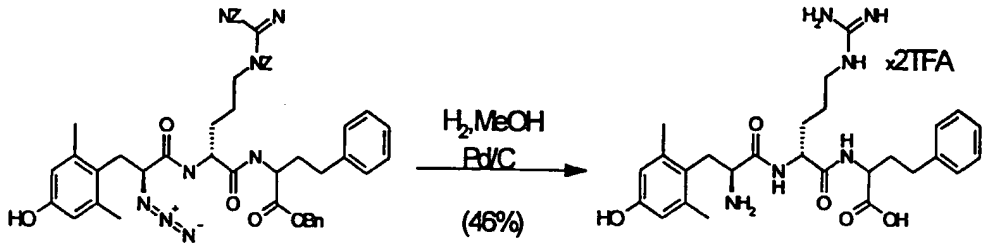



**Procedure:** To a solution of Boc-D-arg-ω,ω'(Z)$_2$-Homophe-OBz TFA salt (226 mg, 0.326 mmol) and azidoacid (96 mg, 0.34 mmol) in DMF (4 mL) was added the 1-hydroxybenzotriazole (HOBt) (66 mg, 0.49 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) (94 mg, 0.49 mmol) at 0 °C. After 30 min at 0 °C, the solution was stirred at rt. for 16 h.The solution was diluted with AcOEt (400 mL) and washed in sequence with saturated $NaHCO_3$ (2x 50 mL), $H_2O$ (1x 50 mL),citric acid (2x 50 mL), $H_2O$ (1x 50 mL), brine (2x 50 mL) and dried over $MgSO_4$. The product was purified by a flash chromatography (AcOEt/Hex, 3:5 to 1:1)(198 mg, 64%)




**Procedure:** To a solution of $Z_2$-derivative (198 mg, 0.207 mmol) in MeOH (10 mL) and one drop of concentrate HCl. After 30 min at reflux, the solution was cooled at r.t.The solution was diluted with AcOEt (400 mL) and washed with saturated $NaHCO_3$ (2x), $H_2O$, brine and dried over $MgSO_4$. The product was purified by a flash chromatography (AcOEt/Hex, 4:6 to 1:1)(115 mg, 61%)

$^1$H NMR ($CDCl_3$):9.51 (1H, broad, NH), 9.37 (1H, broad, NH), 7.55-7.25 (20H, m, H-Ar), 7.03 (1H, d, J=8.0Hz, NH), 6.89 (1H,m, NH), 6.42 (2H, s, H-Ar of DMT), 5.25-5.05 (6H, m, $OCH_2Ph$),4.90 (1H, s, OH), 4.55-4.45 (2H, m, NCH), 3.80 (2H, m), 3.50 (1H, m), 3.46 (1H, dd, J=7.0 Hz, $CH\underline{H}CHN_3$), 2.94 (1H, dd, J=7.0Hz, $C\underline{H}HCHN_3$), 2.51 (2H, m, $CH_2Ph$), 2.21 (6H, s, $CH_3$), 2.05 (1H, m), 1.83 (1H, m), 1.60-1.60 (3H, m), 1.22 (1H, m).




**Procedure:** To a solution of azide (115 mg, 0.126 mmol) in MeOH (3 mL), Pd/C (19.0 mg) and $H_2$ (1atm) was stirred over night. The catalyst was filtered and the solvent was evaporated. The compound was purified by HPLC. (30.5mg, 46%)

$^1$H NMR ($CD_3OD$): 8.52 (1H, d, J=7.5Hz, NH), 7.30-7.15 (5H, m, H-Ar), 6.54 (2H, s, H-Ar of DMT), 4.33 (1H, q,J=5Hz, NCH),4.23 (1H, q,J=5.5Hz, NCH), 3.93 (1H, dd, J=5Hz and 12Hz, NCH), 3.24 (2H, t, J=12.0Hz), 3.10-3.00 (3H, m), 2.80-2.65 (2H, m), 2.29 (6H, s, $CH_3$), 2.17 (1H, m), 1.99 (1H, m), 1.64 (1H, m), 1.44 (1H, m), 1.35-1.20 (2H,m).

EXAMPLE 21 Biological Assays

A. Receptor Affinity - Radioligand Binding Assay

Affinity for μ and δ opioid receptors was assessed *in vitro* using radioligand binding assay employing rat brain membrane preparations as described in Schiller *et al.*, Biophys. Res. Commun., 85, p.1322 (1975) incorporated herein by reference.

Male Sprague-Dawley rats weighing between 350-450g were sacrificed by inhalation of $CO_2$. The rats were decapitated and the brains minus cerebellum were removed and place in ice-cold saline solution and then homogenized in ice-cold 50 mM Tris buffer pH 7.4 (10ml/brain). The membranes were centrifuged at 14000 rpm for 30 min. at 4°C. The pellets were re-suspended in approximately 6ml/brain of ice-cold Tris buffer 50mM pH 7.4 and stored at -78°C until ready for use. Protein quantification of the brain homogenate was conducted according to protein assay kit purchased (Bio-Rad).

($^3$H)- DAMGO and ($^3$H) DAGLE were used as radioligands for the μ and δ receptors, respectively. Radioligand 50 μl, membranes 100 μl and serially diluted test compound were incubated for 1 hr at 22°C. Non specific binding was determined using 500 fold excess of unlabeled ligand in the presence of tracer and membranes. Free ligand was separated from bound by filtration through Whatman GF/B paper (presoaked in polyethylenimine 1% aqueous solution) and rinsing with ice-cold 50mM Tris pH 7.4 using a Brandel cell harvester. The filters were dried and radioactivity was counted in a 24 well microplate in the presence of 500 ml scintillant per well. Radioactivity was measured using a Wallac 1450 Microbeta counter. Inhibition constants ($K_i$) for the various compounds were determined from the $IC_{50}$ according to the Cheng and Prusoff equation.

B. Peripheral Analgesia - PBQ Writhing Assay

PBQ (phenyl-ρ-benzoquinone) induced writhing in mice was used to assess both peripheral analgesia of compounds of the invention according to the experimental protocol described in Sigmund *et al.*, Proc. Soc. Exp. Biol. Med., 95, p. 729(1957) which is incorporated herein by reference. The test was performed on CD #1 male mice weighing between 18 and 22g. The mice were weighed and marked and administered peritoneally with 0.3ml/20g by

weight 0.02% solution of phenylbenzoquinone (PBQ) . The contortions which appeared during a 15 minute time period following the injection were counted and $ED_{50}$ values (dose of compound which induced a 50% reduction in the number of writhes observed compared to the control) was calculated. The PBQ was injected at time intervals of 5, 20 or 60 minutes after subcutaneous or oral administration of the compound (or medium, or standard).

PBQ solution was prepared by dissolving 20mg of PBQ in 5ml ethanol 90% (sigma, reagent, alcohol). The dissolved PBQ was slowly added to 95ml of distilled water continuously shaken and preheated (not boiled). The PBQ solution was left 2 hours before use, and at all times, protected from light. A new solution was prepared every day for the test.

C. Central Analgesia - Hot Plate Assay

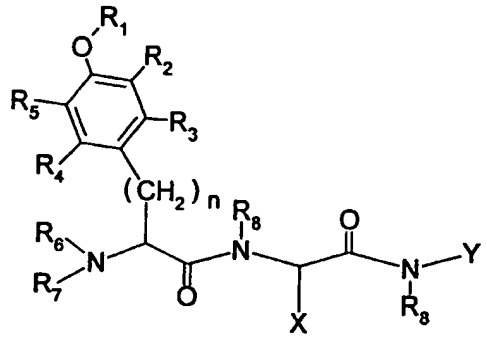
Central analgesic activity was determined by the inhibition of a hot-plate response in mice according to the experimental protocol described in G. Woolfe and A. Macdonald, J. Pharmacol. Exp. Ther., 80, p.300 (1944) which is incorporated herein by reference. CD #1 male mice weighing between 20 and 25g were weighed, marked, and divided into groups of 10. The mice were treated by subcutaneous injection of the compound (or the standard or the medium) in an injection volume equivalent to 0.1 ml/10g p.c. (10ml/kg). The mice were individually evaluated for reaction time on the hot plate at intervals between 15 minutes and 4 hours after administration of compound. The temperature of the hot plate (Sorel, model DS37) was set at 55°C. The mouse was observed for signs of discomfort such as licking or shaking of the paws, attempting to escape (jumping off the plate) or trembling. The reaction time was counted when one of these signs appeared and was noted in "seconds". Mice were limited to

a maximum period of 30 seconds on the plate so as to prevent damage to paw tissue.

For each time reading, the average reaction time of the control group was multiplied by 1.5. The reaction time of each treated mouse was compared to the "control average X 1.5". If the reaction time was inferior to the "control average X 1.5", the mouse was considered to not have had an analgesic effect. If the reaction time was superior to the "control average X 1.5", then the mouse was considered to have had an analgesic effect. The number of analgesic mice in a group determined the analgesic percentage of the compound for this reading. If the analgesic percentage was inferior to 30%, the compound was considered inactive. The $ED_{50}$ (dose of drug required to increase latency of response 2 fold compared to control) was determined by parallel-line probit analysis.

WE CLAIM:

1. The compound represented by formula (I):



(I)

and stereo and optical isomers and racemates, pharmaceutically acceptable salts esters, solvates and hydrates thereof wherein

$R_1$ is selected from H, $C_{1-4}$ alkyl and $C_{1-4}$ acyl;

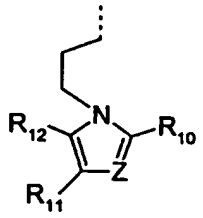
$R_2$ to $R_5$ are independently selected from H, OH, halogen, $C_{1-4}$ alkyl and $C_{1-4}$ alkoxy;

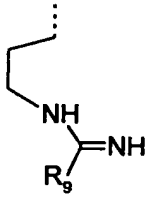
$R_6$ and $R_7$ are independently selected from H and $C_{1-4}$ alkyl;

$R_8$ is H or $C_{1-4}$ alkyl;

n is an integer from 0 to 2;

X is selected from group consisting of (IIa) and (IIb)

IIa 

IIb 

wherein $R_9$ is H, OH, $C_{1-4}$ alkyl, $NH_2$, or $NH-NO_2$; $R_{10}$ to $R_{12}$ are independently H, OH, =O, $NH_2$, $NO_2$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy;

Y is $-CHR_{13}-C(O)-NR_6R_7$, $-CHR_{13}-C(O)-O-R_6$, $-(CHR_{14})_m$-cycloalkyl or $-(CHR_{14})_m$-aryl wherein $R_{13}$ is cycloalkyl, aryl, cycloalkyl-$C_{1-4}$ alkyl or aryl-$C_{1-4}$ alkyl optionally

substituted with OH, halogen, $NR_6R_7$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy and $R_{14}$ is H, OH, halogen, $NR_6R_7$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy, and m is an integer from 0 to 5; and

Z is a heteroatom selected from N, O and S.

2. A compound according to claim 1 wherein $R_1$, $R_2$ and $R_5$ are each H and $R_3$ and $R_4$ are independently selected from H, methyl or methoxy.

3. A compound according to claim 2 wherein $R_3$ and $R_4$ are both methyl.

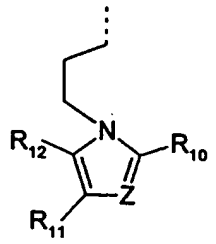
4. A compound according to claim 2 wherein $R_3$ and $R_4$ are both H.

5. A compound according to claim 1 wherein $R_6$, $R_7$ and $R_8$ are independently H or methyl.

6. A compound according to claim 5, wherein $R_6$, $R_7$ and $R_8$ are each H.

7. A compound according to any one of claims 1 to 6, wherein X is the group of formula (IIa):

IIa




wherein Z is selected from N, O and S; and $R_{10}$ to $R_{12}$ are independently H, OH, =O, $NH_2$, $NO_2$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy.

8. A compound according to claim 7, wherein Z is N and $R_{10}$ is H, $NH_2$ or $NO_2$ and $R_{11}$ and $R_{12}$ are independently OH, =O or $C_{1-4}$ alkoxy.

9. A compound according to claim 7, wherein Z is N and $R_{10}$ is H, $NH_2$ or $NO_2$ and $R_{11}$ and $R_{12}$ are both H.

10. A compound according to claim 9, wherein $R_{10}$ to $R_{12}$ are each H.

11. A compound according to claim 7, wherein Y is $-CHR_{13}-C(O)-NR_6R_7$, or $-CHR_{13}-C(O)-O-R_6$ wherein $R_{13}$ is cycloalkyl, aryl, cycloalkyl-$C_{1-4}$ alkyl, or aryl-$C_{1-4}$ alkyl optionally substituted with OH, halogen, $NR_6R_7$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy.

12. A compound according to claim 11, wherein $R_{13}$ is methyl substituted with cyclohexyl or an aryl group selected from phenyl, naphthyl, pyridinyl and quinolinyl optionally substituted with halogen.

13. A compound according to claim 12, wherein said aryl group is phenyl optionally substituted with halogen.

14. A compound according to claim 13, wherein said aryl group is 4-fluoro substituted phenyl.

15. A compound according to claim 7, wherein Y is $-(CHR_{14})_m$-aryl wherein $R_{14}$ is H, OH, halogen, $NR_6R_7$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy and m is 0-5.

16. A compound according to claim 15, wherein m is 1-5, $R_{14}$ is H, OH or $NR_6R_7$ and aryl is phenyl, naphthyl, pyridinyl or quinolinyl optionally substituted with OH, halogen or $C_{1-4}$ alkyl.

17. A compound according to claim 16, wherein m is 3, $R_{14}$ is H or OH and aryl is phenyl.

18. A compound according to claim 17, wherein Y is $-(CH_2)_3$-phenyl.

19. A compound according to claim 17, wherein Y is $-CH_2-CH(OH)-CH_2$-phenyl.

20. A compound according to claim 1, selected from:

2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (1S-carbamoyl-2-phenyl-ethyl)-amide (compound 1);

2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (1S-carbamoyl-2-phenyl-ethyl)-amide (compound 2);
2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-imidazol-1-yl-pentanoic acid (3-phenylpropyl)-amide, (compound 3, and its diastereomers compound 3a , and compound 3b);

2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-(2-nitroimidazol-1-yl)-pentanoic acid (3-phenylpropyl)-amide (compound 4);

2R-[2S-amino-3-(4-hydroxy-phenyl)-propionylamino]-5-(2-amino-imidazol-1-yl)-pentanoic acid (3-phenylpropyl)-amide (compound 5);

2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoic acid (3-phenyl-propyl)amide (compound 6 and its diastereomers compound 6a and compound 6b );

2R-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino],-5-guanidino-pentanoic acid (2-hydroxy-3-phenyl-propyl)amide (compound 7); and

H-Tyr-[D]Arg-Phe-$NH_2$ (compound 8).

2S-{2R-[2S-Amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino] -5-imidazol-1-yl-pentanoylamino}-3-phenyl-propionic acid, trifluroroacetic acid salt (compound 15)

Me-Tyr-D-Arg-Phe-OH (compound 16)

(S)-DMT-(OH)-D-Arg--D-Homophe-$OCH_3$ (compound 17)
(S)-DMT--D-Arg--D-Homophe-OEt (compound 18)

(S)-DMT-(OH)-D-Arg--L-Homophe-$OCH_3$ (compound 19)
2-{2-[2-amino-3-(4-hydroxy-2,6-dimethyl-phenyl)-propionylamino]-5-guanidino-pentanoylamino}-4-phenyl-butyric acid.(compound 20)

21. A pharmaceutical composition comprising a compound according to any one of claims 1 to 20, and a pharmaceutically acceptable carrier, diluent or adjuvant.

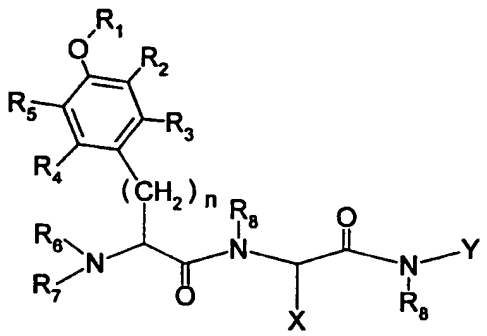
22. A method of inducing analgesia in a mammal comprising administering to said mammal a pharmaceutically effective amount of a compound according to any one of claims 1 to 20.

23. A method of activating opioid receptors in a mammal comprising administering to said mammal an opioid receptor activating amount of a compound according to any one of claims 1 to 20.

24. The use of a compound according to any one of claims 1 to 20 in the manufacture of a medicament for the treat of pain.

25. A pharmaceutical formulation for use in the treatment of pain, wherein the active ingredient is a compound according to any one of claims 1 to 20.

26. A process for preparing compounds of formula (I),



(I)

and pharmaceutically acceptable salts thereof wherein

$R_1$ is selected from H, $C_{1-4}$ alkyl and $C_{1-4}$ acyl;

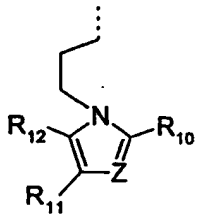
$R_2$ to $R_5$ are independently selected from H, OH, halogen, $C_{1-4}$ alkyl and $C_{1-4}$ alkoxy;

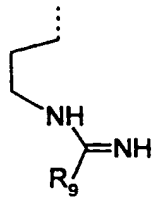
$R_6$ and $R_7$ are independently selected from H and $C_{1-4}$ alkyl;

$R_8$ is H or $C_{1-4}$ alkyl;

n is an integer from 0 to 2;

X is selected from group consisting of (IIa) and (IIb)

IIa 

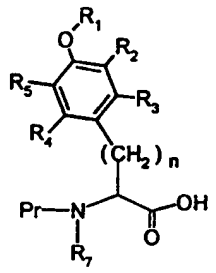
IIb 

wherein $R_9$ is H, OH, $C_{1-4}$ alkyl, $NH_2$, or NH-$NO_2$; $R_{10}$ to $R_{12}$ are independently H, OH, =O, $NH_2$, $NO_2$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy;

Y is $-CHR_{13}-C(O)-NR_6R_7$, $-CHR_{13}-C(O)-O-R_6$, $-(CHR_{14})_m$-cycloalkyl or $-(CHR_{14})_m$-aryl wherein $R_{13}$ is cycloalkyl, aryl, cycloalkyl-$C_{1-4}$ alkyl or aryl-$C_{1-4}$ alkyl optionally substituted with OH, halogen, $NR_6R_7$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy and $R_{14}$ is H, OH, halogen, $NR_6R_7$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy, and m is an integer from 0 to 5; and

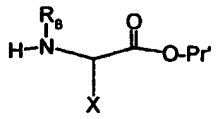
Z is a heteroatom selected from N, O and S;

comprising coupling a compound of formula (i)



i

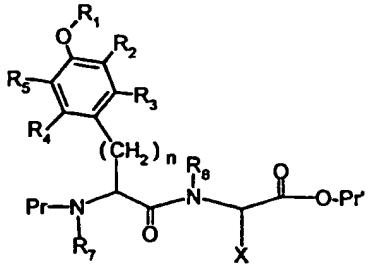
wherein Pr is an amino-protecting group, with a compound of formula (ii)



ii

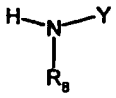
wherein Pr' is a carboxyl-protecting group,

to give intermediate of formula (iv)



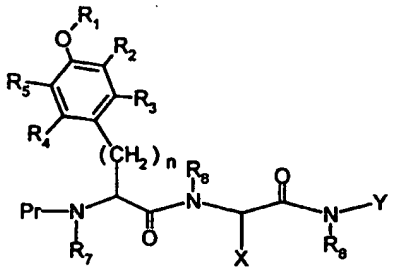
iv ;

removing carboxyl-protecting group Pr' and then coupling intermediate (iv) with a compound of formula (iii)



iii

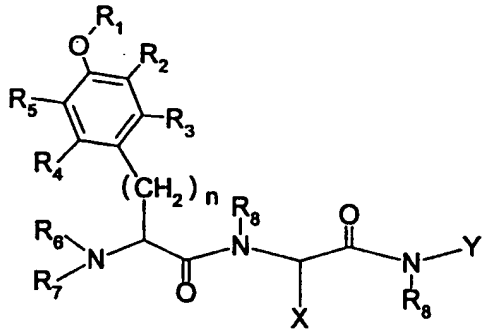
to give an intermediate of formula (v)



v

27. A process according to claim 26, further comprising removing amino-protecting group Pr from intermediate (v) to give a compound of formula (I).

28. A process for preparing compounds of formula (I)



(I)

and pharmaceutically acceptable salts thereof wherein

$R_1$ is selected from H, $C_{1-4}$ alkyl and $C_{1-4}$ acyl;

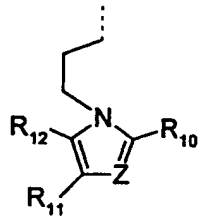
$R_2$ to $R_5$ are independently selected from H, OH, halogen, $C_{1-4}$ alkyl and $C_{1-4}$ alkoxy;

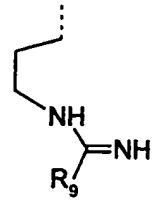
$R_6$ and $R_7$ are independently selected from H and $C_{1-4}$ alkyl;

$R_8$ is H or $C_{1-4}$ alkyl;

n is an integer from 0 to 2;

X is selected from group consisting of (IIa) and (IIb)

IIa 

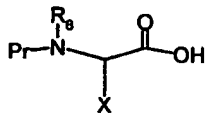
IIb 

wherein $R_9$ is H, OH, $C_{1-4}$ alkyl, $NH_2$, or $NH-NO_2$; $R_{10}$ to $R_{12}$ are independently H, OH, =O, $NH_2$, $NO_2$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy;

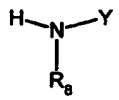
Y is $-CHR_{13}-C(O)-NR_6R_7$, $-CHR_{13}-C(O)-O-R_6$, $-(CHR_{14})_m$-cycloalkyl or $-(CHR_{14})_m$-aryl wherein $R_{13}$ is cycloalkyl, aryl, cycloalkyl-$C_{1-4}$ alkyl or aryl-$C_{1-4}$ alkyl optionally substituted with OH, halogen, $NR_6R_7$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy and $R_{14}$ is H, OH, halogen, $NR_6R_7$, $C_{1-4}$ alkyl or $C_{1-4}$ alkoxy, and m is an integer from 0 to 5; and

Z is a heteroatom selected from N, O and S;

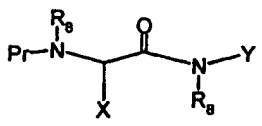
comprising coupling a compound of formula (ii')

 ii'

wherein Pr is an amino-protecting group, with a compound of formula (iii)

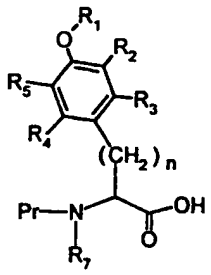
 iii

to give intermediate of formula (iv')



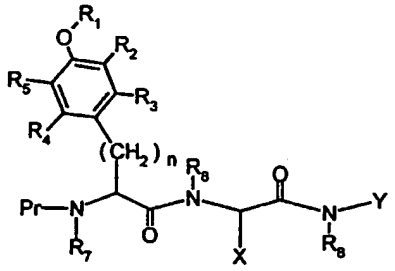
iv'

removing amino-protecting group Pr and then coupling intermediate (iv') with a compound of formula (i)



i

to give an intermediate of formula (v)



v

29. A process according to claim 28, further comprising removing amino-protecting group Pr from intermediate (v) to give a compound of formula (I).

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00826

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 5/087, A61K 38/06
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REGISTRY, CAPLUS, WPI

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP 0755942 A1 (DAIICHI PHARMACEUTICAL CO., LTD.), 29 January 1997 (29.01.97) -- | 1-21,24-29 |
| A | WO 9707130 A1 (ASTRA AKTIEBOLAG), 27 February 1997 (27.02.97) -- | 1-21,24-29 |

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 31 August 1998 | 03 -09- 1998 |
| Name and mailing address of the ISA/<br>Swedish Patent Office<br>Box 5055, S-102 42 STOCKHOLM<br>Facsimile No. +46 8 666 02 86 | Authorized officer<br>Solveig Gustavsson<br>Telephone No. +46 8 782 25 00 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00826

**Box I** Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 22–23
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

27/07/98

International application No. PCT/SE 98/00826

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| EP 0755942 A1 | 29/01/97 | FI 963568 A | 07/11/96 |
| | | JP 2275181 A | 09/11/90 |
| | | JP 2678660 B | 17/11/97 |
| | | NO 963791 A | 11/11/96 |
| | | CA 2185212 A | 14/09/95 |
| | | CN 1148392 A | 23/04/97 |
| | | JP 7300496 A | 14/11/95 |
| | | WO 9524421 A | 14/09/95 |
| WO 9707130 A1 | 27/02/97 | AU 6760096 A | 12/03/97 |
| | | EP 0845003 A | 03/06/98 |
| | | NO 980592 A | 11/02/98 |
| | | SE 9502877 D | 00/00/00 |
| | | SE 9503924 D | 00/00/00 |